

Despommier's

Parasitic Diseases

Griffin
Gwadz
Hotez
Knirsch



Eighth Edition

Parasites Without Borders, Inc. NY

Dickson D. Despommier, Daniel O. Griffin, Robert W.
Gwadz, Peter J. Hotez, Charles A. Knirsch

Life Cycles by
John Karapelou

Photographs by
Dickson D. Despommier
and Daniel O. Griffin

Despommier's Parasitic Diseases

Eighth Edition

>400 illustrations in full color

>4,000 references

Parasites Without Borders, Inc. NY

Daniel O. Griffin, M.D., Ph.D. CTropMed® ISTM CTH© Department of Medicine-Division of Infectious Diseases, Columbia University Irving Medical Center New York, New York, NY 10032.

Robert W. Gwadz, Ph.D. Captain USPHS (ret), Visiting Professor, Collegium Medicum, The Jagiellonian University, Krakow, Poland, Fellow of the Hebrew University of Jerusalem, Fellow of the Ain Shams University, Cairo, Egypt, Chevalier of the Nation, Republic of Mali.

Peter J. Hotez, M.D., Ph.D., FASTMH, FAAP, Dean, National School of Tropical Medicine, Professor, Pediatrics and Molecular Virology & Microbiology, Baylor College of Medicine, Texas Children's Hospital Endowed Chair of Tropical Pediatrics, Co-Director, Texas Children's Hospital Center for Vaccine Development, Baker Institute Fellow in Disease and Poverty, Rice University, University Professor, Baylor University, former United States Science Envoy.

Charles A. Knirsch, M.D., M.P.H. Founding Director Parasites Without Borders, Inc.

A number of the drawings utilized herein are printed with the permission of Karapelou Medical Art, with all rights reserved.

Cover Design: Daniel O. Griffin (2025)

Page layout and design: Daniel O. Griffin and Ines Knirsch

Library of Congress Cataloguing-in-Publication Data

Despommier's Parasitic Diseases / Daniel O. Griffin, Peter J. Hotez, Charles A. Knirsch:
- 8th edition

p. cm.

Includes bibliographical references and index

ISBN :978-0-9978400-8-7 (Hardcover)

ISBN:ISBN: 978-0-9978400-9-4 (Paperback version)

1. Parasitic diseases / Daniel O. Griffin, Robert W. Gwadz, Peter J. Hotez, Charles A. Knirsch.

IV. Title. Parasitic Diseases

©

© 2025 Parasites Without Borders; 2019 Parasites Without Borders, 2017 Parasites Without Borders, 2005, 2000 Apple Tree Productions; 1995, 1989, 1982, Springer-Verlag, New York, Inc.

All rights reserved. This work may not be translated or copied in whole or in part without the written permission of Parasites Without Borders (www.parasiteswithoutborders.com) except for small portions quoted in reviews or academic works. Any other use (electronic information storage, retrieval, or adaptation, computer software, or by similar or dissimilar methodology) is strictly prohibited. Our use of names, trade names, or trademarks in Parasitic Diseases 8th ed., even if they are not identified as such, must not be interpreted to mean that such names, as defined by the Trade Marks and Merchandise Marks Act, may therefore be used by anyone else. Neither the authors nor the publisher accept legal responsibility for any errors or omissions that may be made. The publisher makes no warranty regarding the material contained herein.

We dedicate this 8th edition to Dickson Despommier.

Dr. Dickson Despommier spent 38 years as a professor of microbiology and public health in environmental health sciences at Columbia University, where he won the Best Teacher Award six times. In 2003, he received the American Medical Student Association's National Golden Apple Award for Teaching Excellence. His work on vertical farms was featured on such top national media as the BBC, French national television, CNN, The Colbert Report, and The Tonight Show, as well as full-length articles in The New York Times, Times magazine, Scientific American, and The Washington Post. He spoke at the TED Conference, PopTech, and the World Science Festival, and was invited by the governments of China, India, Mexico, Jordan, Brazil, Canada, and South Korea to work on their environmental problems. He was invited to speak at numerous national and international professional annual meetings as a keynote speaker, and at universities, including Harvard and MIT. He was one of the visionaries featured at the Museum of Science and Industry, Chicago.

The world lost a great mentor, friend, and scientist on February 7th, 2025.



Dickson D. Despommier

Acknowledgements:

We acknowledge the contribution of John Karapelou for his elegant life cycle drawings. Thanks to David Scharf for granting us use of his stunning scanning electron micrographs of the very organisms, organelles and cellular environments that attract legions of new students to the field of tropical medicine. Thanks to all the course directors of parasitic diseases and parasitology who choose our book to guide their students through the complexities of life cycles, clinical presentations, and biology of parasites. We hope that the 8th edition proves even more useful for you and your students in the coming years.

Preface

We continue our mission to present the newest medically relevant findings regarding protozoal and helminth parasites. Since the first edition, the authors have endeavored to describe the interrelationship of parasitic diseases, arthropod vectors, and environmental factors mitigating or exacerbating public health and clinical disease in humans and selected livestock. Today, this is often referred to as One World Health and many organizations have embraced work to this end. In the fifth Edition, we noted that the Millennium Development Goals set aspirational targets that could impact human health within countries endemic to many diseases covered in this textbook. In 2020, The World Health Organization (WHO) released *Ending the Neglect to Attain the Sustainable Development Goals: A Road Map for Neglected Tropical Diseases 2021–2030*, which sets revised targets and critical performance indicators for managing neglected tropical diseases (NTD) to advance control and elimination work by 2030.¹ Since the first road map was released in 2010, 600 million fewer people require interventions for NTD's such as mass drug treatment or surgery for disabling disease morbidity. Many individual disease elimination programs are well underway, and over 40 countries have certified them as eliminating at least one disease. Now, more than ever, coordination across programs in countries is essential where multiple diseases are co-endemic. This includes developing the evidence to safely co-administer medicines, integrated reporting of adverse safety events, and training healthcare workers to manage the integrated control efforts. In the 8th Edition of *Parasitic Diseases*, we have expanded the foundation and knowledge base for which scientists can learn and join the effort to contribute to mitigating parasitic diseases, including many diseases destined for elimination in the WHO NTD roadmap. New features to the 8th edition include an update on the epidemiology of the diseases, efforts underway to discover and develop new medicines and vaccines, and the status of disease management and elimination programs. *Parasites Without Borders* offers the latest PDF version of ***Parasitic Diseases*** available to the world-at-large free of charge.

The number of eukaryotic parasites whose entire genomes have been available since the completion of The Human Genome Project in 2003 continues to grow, and they are featured in our 8th edition. Genomic datasets hold great promise for developing new vaccines, drugs, and control programs based on identifying unique molecular pathways essential to each pathogen. These ongoing projects are a testament to the dedication and creativity of highly trained scientists whose relentless collective efforts help stem the spread of these debilitating infectious diseases.

Progress in the rapid identification of parasites by targeting their genomic signatures in biological specimens has dramatically improved and simplified their laboratory diagnosis. It is now the preferred approach in most hospitals throughout the developed world. While molecular-based testing has essentially replaced the microscope as the preferred method of diagnosing parasitic infections in most laboratories, in the less developed world, microscopically identifying them remains the readily accessible technology. At the same time, many new capacity-building programs attempt to scale up current technologies.

That is why we continue to include a diagnostic atlas in our text.

Recent advances have greatly improved our understanding of the molecular mechanisms underlying some parasite's ability to interfere with signaling pathways. We now have much more detailed datasets regarding these complex parasite survival mechanisms. Unraveling the pathways by which parasites engineer their survival in the face of our highly evolved immune system has also provided clues in treating illnesses unrelated to the parasites themselves. "Swords into plowshares" molecular strategies provide the basic science for "parapharmaceuticals" research for coronary artery disease, stroke, clotting disorders, various autoimmune conditions, and many more human diseases.²

Numerous newly discovered novel compounds reflect cutting-edge scientific advances in drug discovery and design using high-throughput screening algorithms, cryo-electron microscopy, and virtual reality visualization technologies. Many are now being tested in clinical trials. These approaches have already provided clinicians with a new generation of drugs, many with fewer harmful side effects than the ones they replaced. Controlling parasite populations at the community level is an anticipated consequence of such developments without endangering the health of those we wish to help.

The remarkable success of programs targeting river blindness and Guinea worm, both of which have been controlled and essentially eliminated in many countries, is proof of concept that by applying the principles of public health, we can rid the world of pathogens that cause severe diseases besides the well-known examples of smallpox and polio. Similarly, the Southern Cone initiative of South America has succeeded in dramatically reducing cases of Chagas disease. In Africa, as of 2019, there have been less than 1,000 cases of African human trypanosomiasis annually.

High morbidity and mortality rates throughout the tropics are still due to some of the most commonly occurring parasitic infections, most notably malaria. While no new classes of drugs for treating resistant malaria are commercially available, artemisinin derivatives still effectively reduce the mortality of the world's most devastating infectious disease wherever that chemotherapeutic agent is available. Distribution of the latest effective malaria vaccine, RTS, S/AS01, is ongoing and should result in 18 million doses given to 12 African countries by the end of 2025, according to the WHO.³ A second malaria vaccine, R21/Matrix-M, was recommended for approval by WHO in October 2023, bringing yet another positive intervention to improving survival in children under five.⁴ Worm infections have exacted their toll on humanity for millennia, with children as their primary victims. Until basic sanitation is implemented, this will continue to be the norm for most underdeveloped countries and impoverished communities.

Diarrheal diseases caused by a variety of infectious agents, including *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, and *Cyclospora cayetanensis* round out the list of infectious diseases that those living in poverty in the less developed world must continue to endure. Roughly 50 percent of the world's farmers rely on untreated feces and urine as their only source of fertilizer. Education on applying basic

sanitation to render human waste into safe-to-use manure remains high on global public health priorities.

Political instability of vast regions of Africa and the Middle East has catalyzed the re-emergence of many infectious diseases. This is primarily due to environmental destruction, abandonment of control programs, and forced migration of thousands of individuals from relatively safe regions to places no one should have to occupy, no matter how short the duration. According to WHO, as of September 30, 2023, the number of refugees worldwide was estimated to be over 108 million. The impact of the COVID-19 pandemic on vaccine and disease elimination programs is only now being quantified with mitigation efforts underway. These seemingly intractable situations require more than vaccines and drugs to affect a cure. Social stability, equity, economic development, and long-term planning need to go hand in hand with strengthening the universal health system, which is underway today.

Through educating students, clinicians, and parasitologists and vigilance of commitment, we can improve the lives of hundreds of millions of less fortunate individuals by helping them live longer and lead more productive lives. The 8th edition of *Parasitic Diseases* is dedicated to this premise. Please join us in this global effort. Your help is critical to the success of combating these neglected infectious diseases.

Parasites without borders short:

Parasites Without Borders was founded as a direct response to the question: “What can I do to help eliminate human suffering due to parasitic infections?” For us, the choice was easy: more and better education for all those in a position to apply medical knowledge directly to populations needing relief from the burden of parasitic diseases. The three founders have a lifetime of experience teaching parasitic diseases to medicine students, both within the U.S.A. and abroad. Our mission statement remains unshakably clear: we will continue our goal of bringing the latest medical and basic biological information about diseases caused by eukaryotic parasites to every clinician and student throughout the world, gratis. Please visit our website at:

<http://www.parasiteswithoutborders.com>

References

1. Organization, W. H., Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva. *Licence: CC BY-NC-SA 3.0 IGO 2020*.
2. Despommier, D., *People, Parasites, and Plowshares*. 2014.
3. WHO 18 million doses of first-ever malaria vaccine allocated to 12 African countries for 2023–2025: Gavi, WHO and UNICEF. <https://www.who.int/news/item/05-07-2023-18-million-doses-of-first-ever-malaria-vaccine-allocated-to-12-african-countries-for-2023-2025--gavi--who-and-unicef> (accessed June 2).
4. WHO WHO recommends R21/Matrix-M vaccine for malaria prevention in updated advice on immunization. <https://www.who.int/news/item/02-10-2023-who-recommends-r21-matrix-m-vaccine-for-malaria-prevention-in-updated-advice-on-immunization> (accessed June 2).



Daniel O. Griffin



Robert W. Gwadz



Peter J. Hotez



Charles A. Knirsch

This Week in Parasitism



This Week in Parasitism (TWiP) is a podcast about eukaryotic parasites started by Vincent R. Racaniello and Dickson D. Despommier. When Daniel O. Griffin, MD joined the team in January 2015 he added his expertise in infectious diseases and a new feature of TWiP: the case study. Each week Daniel presents the symptoms and signs of an interesting case that he has investigated during his work, without identifying the infectious agent. Listeners are encouraged to send in their guesses to this weekly infectious disease mystery. We call this new change ‘TWiP reboot’.

The TWiP trio was later joined by Christina Naula, from the University of Glasgow. TWiP provides an informative conversation about parasites which is accessible to everyone, no matter what their science background. As science Professors at Columbia University, Dickson and Vincent have directed research laboratories focused on parasites and viruses. Their enthusiasm for teaching inspired them to reach beyond the classroom with this media. TWiP is for everyone who wants to learn about parasites in an informal way.

Find us on iTunes, download us with your favorite pod-catcher or go to our website.

<http://www.microbe.tv/twip>

Contents

I. Acknowledgements	iv
II. Preface	v-ix
III. Eukaryotic Parasites	1-8
IV. The Protozoa	9-10
1. <i>Giardia lamblia</i>	11-21
2. Introduction to the <i>Leishmania</i> species	22-29
3. Cutaneous Leishmaniasis	30-40
<i>Leishmania (L.) major</i>	
<i>Leishmania (L.) tropica</i>	
<i>Leishmania (L.) mexicana</i>	
4. Mucocutaneous Leishmaniasis	41-49
<i>Leishmania (V.) braziliensis</i>	
5. Visceral Leishmaniasis	50-60
<i>Leishmania (L.) donovani</i>	
<i>Leishmania (L.) infantum</i>	
<i>Leishmania (L.) infantum chagasi</i>	
6. African Trypanosomiasis	61-74
<i>Trypanosoma brucei rhodesiense</i>	
<i>Trypanosoma brucei gambiense</i>	
7. American Trypanosomiasis	75-88
<i>Trypanosoma cruzi</i>	
8. <i>Trichomonas vaginalis</i>	89-97
9. The Malarias	98-128
<i>Plasmodium falciparum</i>	
<i>Plasmodium vivax</i>	
<i>Plasmodium ovale</i>	
<i>Plasmodium malariae</i>	
<i>Plasmodium knowlesi</i>	
10. <i>Cryptosporidium parvum</i>	129-138
11. <i>Toxoplasma gondii</i>	139-152
12. <i>Entamoeba histolytica</i>	153-166
13. <i>Balantidium coli</i>	167-172
14. Other Protozoa of Medical Importance	173-192
<i>Babesia</i> spp.	
<i>Cystoisospora belli</i>	
<i>Cyclospora cayetanensis</i>	
<i>Naegleria fowleri</i>	
<i>Acanthamoeba</i> spp.	
<i>Balamuthia mandrillaris</i>	
<i>Blastocystis hominis</i>	
<i>Dientamoeba fragilis</i>	

15. Non-pathogenic Protozoa	193-196
Commensal Flagellates	
Commensal amoebae	
V. The Nematodes	197-199
16. <i>Enterobius vermicularis</i>	200-206
17. <i>Trichuris trichiura</i>	207-215
18. <i>Ascaris lumbricoides</i>	216-225
19. The Hookworms	226-240
<i>Necator americanus</i>	
<i>Ancylostoma duodenale</i>	
<i>Ancylostoma ceylanicum</i>	
20. <i>Strongyloides stercoralis</i>	241-252
21. <i>Trichinella spiralis</i>	253-264
22. Lymphatic Filariae	265-277
<i>Wuchereria bancrofti</i>	
<i>Brugia malayi</i>	
23. <i>Onchocerca volvulus</i>	278-289
24. <i>Loa loa</i>	290-297
25. <i>Dracunculus medinensis</i>	298-303
26. Other Nematodes of Medical Importance	304-311
<i>Capillaria hepatica</i>	
<i>Capillaria philippinensis</i>	
<i>Dirofilaria immitis</i>	
<i>Mansonella ozzardi</i>	
<i>Mansonella perstans</i>	
<i>Mansonella streptocerca</i>	
<i>Oesophagostomum bifurcum</i>	
<i>Ternidens diminutus</i>	
27. Aberrant Nematode Infections	312-327
Cutaneous Larva Migrans	
Visceral Larva Migrans	
VI. The Cestodes	328-330
28. <i>Taenia saginata</i>	331-338
29. <i>Taenia solium</i>	339-353
30. <i>Diphyllobothrium latum</i>	354-361
31. Other Tapeworms of Medical Importance	362-371
<i>Hymenolepis nana</i>	
<i>Hymenolepis diminuta</i>	
<i>Dipylidium caninum</i>	

32. Juvenile Tapeworm infections of Humans	372-389
<i>Echinococcus granulosus</i>	
<i>Echinococcus multilocularis</i>	
<i>Mesocestoides</i> spp.	
<i>Spirometra</i> spp.	
<i>Taenia</i> spp. (other than <i>saginata</i> and <i>solium</i>)	
VII. The Trematodes	390-392
33. The Schistosomes	393-421
<i>Schistosoma mansoni</i>	
<i>Schistosoma japonicum</i>	
<i>Schistosoma haematobium</i>	
<i>Schistosoma mekongi</i>	
<i>Schistosoma intercalatum</i>	
34. <i>Clonorchis sinensis</i> and <i>Opisthorchis</i> spp.	422-429
35. <i>Fasciola hepatica</i>	430-437
36. <i>Paragonimus westermani</i>	438-445
37. Other Trematodes of Medical Importance	446-455
<i>Fasciolopsis buski</i>	
<i>Echinostoma</i> spp.	
<i>Heterophyes heterophyes</i>	
<i>Metagonimus yokogawai</i>	
<i>Nanophyetes salmincola</i>	
VIII. The Arthropods	456-457
38. The Insects	458-497
39. The Arachnids	498-517
40. Other Arthropods of Medical Importance	518-521
Appendix A. Procedures for Collecting Clinical Specimens for Diagnosing Protozoan and Helminthic Parasites	522-525
Appendix B. Laboratory Diagnostic Methods	526-533
Appendix C. Diagnostic Color Atlas	534-551
Parasite Identification Charts	552-555
Clinical Appendix	556-595
Pronunciation Guide	596-601
Acronyms	602-605
Index	606-610



Parasites Without Borders was founded as a direct response to the question: “What can I do to help eliminate human suffering due to parasitic infections?” For us the choice was easy; more and better education for all those in a position to apply medical knowledge directly to populations in need of relief from the burden of parasitic diseases. The three founders have a lifetime of experience in teaching parasitic diseases to students of medicine, both within the U.S.A. and abroad. Our mission statement is clear; we want to help bring the latest medical and basic biological information pertaining to diseases caused by eukaryotic parasites to every clinician and student throughout the world.

<http://www.parasiteswithoutborders.com>

III. Eukaryotic Parasites

Eukaryotic parasites comprise subsets of organisms within the protozoan and helminth (parasitic worm) groups. Medically relevant arthropods have also been included in discussions of eukaryotic parasites since so many pathogens are transmitted to humans by arthropod vectors. Some arthropods cause disease on their own.

From a biological perspective, a phylogenetic presentation of eukaryotic parasitic organisms would satisfy specialists who strictly adhere to the zoological literature. Most medical students and practicing clinicians would have little interest in taxonomic information. Physicians are inclined to group them according to their syndromes if they were to classify them at all. We have settled upon a compromise in which the reader encounters these organisms in a somewhat biologically correct order, with an outline of their classification and clinical presentations. It is intellectually satisfying to review parasitic organisms with a nod to their evolutionary importance, allowing each student to learn about them in a sequence most parasitologists would be happy with, going from single-cell organisms to helminths and beyond. We present protozoans first, followed by the helminths, and finally, complete the synopsis with medically relevant arthropods.

The following sections are organized to enable the student or clinician easy access to a highly distilled body of information. This relates to the general schemes employed when these organisms interact with the human host to produce disease. Rather than being an exhaustive text, only biological information essential to the understanding of clinical aspects of a given disease-causing organism will be emphasized. The following topics are deemed medically relevant: 1. mechanisms

of entry, 2. niche selection, 3. reproduction, 4. survival mechanisms (i.e., virulence factors), and 5. mechanisms of pathogenesis.

The last fifty years have been remarkable for the community-based control of pathogenic organisms. New vaccines and antibiotics have also helped reduce the incidence of numerous pathogenic microorganisms. At the same time, the emergence and re-emergence of a broad spectrum of infectious agents have occurred including viruses (e.g., SARS CoV 1, SARS CoV2, HIV, monkeypox, avian influenza, dengue, chikungunya, Zika), bacteria (e.g., *Legionella pneumophila*, *Borrelia burgdorferi*, *Escherichia coli* strain OH157), protozoa (e.g., *Cryptosporidium parvum*, *Cyclospora cayetanensis*), and helminths (e.g., *Echinococcus multilocularis*, *Angiostrongylus cantonensis*, *Trichinella spiralis*). Viewed from an evolutionary perspective, humans represent a highly successful system of essential niches, of which an astonishingly wide variety of eukaryotes have taken advantage.

It is difficult to comprehend the prevalence of and suffering caused by eukaryotic parasites. This is especially true when hearing a large number, as is the case for *Ascaris lumbricoides*, which infect hundreds of millions worldwide. When one hears for the first time that close to 250 million people are infected with malaria each year, and around 500,000 children per year die in Africa alone from this infection, these facts seem abstract. But, when a single child suffering from the cerebral form of malaria is admitted into a modern hospital in critical condition, and, regardless of treatment, that young person dies, the healthcare community of that institution goes into collective shock. If the death occurred at a teaching hospital, a grand round is the usual outcome, to provide a potential teaching opportunity, and to see if anything could have been

done to spare that life. Unfortunately, the most lethal malaria species, *Plasmodium falciparum*, is becoming increasingly resistant to the medications in our arsenal.

Parasitic Protozoa

What is a protozoan? Which ones cause disease? How do parasitic protozoa differ from their free-living counterparts? What is the pathogenic mechanism(s) by which they cause disease? Over 200,000 known species of single-celled organisms fall under the category of protozoa, while many more, no doubt, await discovery. Only a tiny fraction of these are parasitic on the human host; some cause significant harm, especially when encountered for the first time.

Protozoans are single-cell organisms inside of which usually reside one membrane-bound nucleus, with a few exceptions, such as *Giardia lamblia* and *Dientamoeba fragilis*. Most protozoa have one type of organelle that aids their movement (e.g., flagella, undulating membrane, cilia). Metabolic pathways vary from group to group, with anaerobic and aerobic energy metabolisms among the parasites to be discussed. As is the case for all parasitic organisms, the host provides the energy source. There are a variety of drugs that take advantage of the dependence of parasites on host energy metabolism.

All single-cell organisms have complex biochemistries, often employing unique pathways that give some of them remarkable evolutionary advantages. These include the ability of a given population to vary their protein surfaces, edit their mRNA transcripts, secrete peptides that prevent the fusion of lysosomal membranes to the parasitophorous vacuole, and secrete compounds that inhibit host protective immune responses. A plethora of unique molecular pathways have been described for this diverse group of parasites,

but a comprehensive description of them is beyond the scope of this book. Attention will be paid to the biochemical and molecular biological pathways that relate to understanding the mechanisms of pathogenesis or parasite survival strategies.

In addition, a few free-living protozoa become pathogenic when they accidentally enter a human host, and a few of these facultative parasites are lethal.

Mechanisms of Entry

Protozoans enter their host in one of several routes: oral, sexual intercourse, inhalation, direct contact, and through the bites of blood-sucking vectors. Avoidance or prevention of infection requires an intimate knowledge of its transmission cycle, and knowing the route of entry into the host is one of the most critical aspects.

Parasitic protozoa have evolved stages that facilitate their dispersal into the environment, increasing their chances of encountering a host. Some intestinal protozoa produce a resistant cyst, leaving them dormant in the external environment for long periods, months to years, in some cases. Others depend upon human activities for their dispersal, as in the case of *Trichomonas vaginalis*, which is sexually transmitted. Some amoebae infect humans through inhalation or direct contact.

Vector-borne organisms rely on the biology of blood-sucking insects, for the most part. Mosquitoes transmit all malarial species (*Plasmodium* spp.), tsetse flies transmit African Sleeping Sickness (*Trypanosoma brucei* spp.), and sandflies transmit all species of *Leishmania*. In these instances, the organism is injected directly into the host's bloodstream or interstitial tissue fluids, where it undergoes complex development once it achieves its essential niche.

Trypanosoma cruzi, an organism transmitted by the reduviid bug, employs a more complex strategy. The infectious organisms are excreted along with the fecal exudate when the insect takes its second blood meal. Humans unwittingly become infected by rubbing the organisms in the insect's feces into the bite wound or a mucous membrane after the reduviid withdraws its mouthparts.

Niche Selection

Each protozoan has been selected for life in a specific essential niche, which can only be defined by a comprehensive knowledge of that site's anatomical, physiological, and biochemical features. Consider the difficulties associated with describing the essential niche, be it that of a parasite or any other organism. The intracellular milieu of the average red blood cell represents one of the best studies of all intracellular environments. However, we still do not understand how an anucleate cell's membranes interact with vascular endothelial cells when the red cell enters the capillary and exchanges gases with the surrounding tissues. A red blood cell infected with *P. falciparum* behaves quite differently from a normal one, failing to deform as it enters the capillary. This single aspect of the infection has pathological consequences for the host, as will be detailed in the section dealing with the clinical aspects of malaria.

The infected red cell's internal molecular environment must be considered a "hybrid," consisting of host and parasite elements. Proteins produced by the developing merozoite are located in the host cell's cytoplasm; some proteins even integrate into the red cell membrane surface, forming complexes with host structural proteins such as spectrin and glycophorin. Others remain in the general region of the red cell cytoplasm. Over the entire malarial parasite's developmental cycle, new proteins locate to specific regions of an ever-

changing host cell (i.e., essential niche) environment. The infected red cell represents a dynamic situation; even with the most sophisticated instrumentation, it has been impossible to fully appreciate the setting where and how this important pathogen lives out its life.

To complicate matters even further, no two species of *Plasmodium* behave the same in their erythrocytic niche. We may never gain a "full face-on" view of this or any other pathogen to sufficiently design new therapeutics that would prevent the organism from taking full advantage of its ecological setting. The complexities presented to the research parasitologist by just this single organism continue to challenge them to design innovative experiments that may allow us one day more than a glimpse into its sequestered intracellular life.

At the other end of the scale is *Toxoplasma gondii*, a generalist protozoan capable of infecting and reproducing within any cell in any mammalian host. *T. gondii*'s lack of host specificity makes it the most widely distributed parasite worldwide.

Migration to favorable sites within the host often requires an active role for the pathogen, but frequently, they "hitch a ride" in our bloodstream or through our intestinal tract. Some protozoan parasites can infect cells that, under most circumstances, would protect us from these organisms. The macrophage is a permissive host cell for *T. gondii* and all *Leishmania* species. In these infections, the very cell type we depend upon for innate protection against invaders becomes the culprit, aiding their dispersal throughout our body.

Division and Reproduction

Multiplication within the human host is the rule for protozoans, unlike most helminth infections, which usually result in a single adult parasite. The definitive host is the one

harboring the sexual or adult stages of a given parasite. Humans are not the definitive host for many protozoan infections, including the *Plasmodium* spp. and *Toxoplasma gondii*. Female anopheline mosquitoes are the definitive hosts for all malaria species infecting humans, while the domestic cat and other Felidae are definitive hosts for the sexual stages of *Toxoplasma*. Humans are the definitive host for *Cryptosporidium parvum*. It should be emphasized that not all parasitic protozoa have sexual cycles.

All protozoans reproduce asexually after gaining entrance into the human host. Pathological consequences result directly from their increasing numbers. They place ever-increasing demands upon their essential niches during the height of the infection. The mechanisms by which protozoa divide asexually are numerous, with binary fission being the most common. Malarial parasites reproduce within the red cell by schizogony, in which the organism undergoes nuclear division within a common cytoplasm (karyokinesis). Just before rupturing out of the hemoglobin-depleted red cell, the parasite's cytoplasm divides to accommodate each nucleus, leaving its toxic waste product, hemozoin crystals, in the now empty red cell stroma. Protozoan parasites reproduce in their insect vectors (intermediate hosts) entirely differently from their mammalian hosts.

Mechanisms of Survival

Each pathogenic protozoan species has been selected for life as a parasite by evolving strategies that 1. inhibit or divert our immune system, 2. avoid or inhibit intracellular killing mechanisms, and 3. infect regions of the body incapable of protective immune responses. For example, the African trypanosomes produce "smoke screens" of surface antigens whose sole purpose is to keep the immune system busy. At the same time, a small select

population changes its protein coat to a different antigenic variant, thus temporarily escaping the host's immune surveillance system. Specific stages of the malarial parasite and *Giardia lamblia* can also vary their surface proteins, presenting our immune system with a bewildering array of antigenic determinants to deal with as infection progresses. *T. gondii* inhibits the fusion of lysosomal vesicles with the parasitophorous vacuole, thus escaping the killing effects of acid hydrolases. *Cryptosporidium parvum* and all species of malaria occupy immunologically "silent" niches. *Trypanosoma cruzi* escapes the parasitophorous vacuole, penetrating naked cytoplasm and hiding in plain sight. There are numerous other examples, and they will be discussed whenever relevant. Independent of the biochemical strategy employed by the protozoan parasite, the result is tissue damage, often severe.

Mechanisms of Pathogenesis

Regardless of the mechanisms employed by the parasite to escape being killed, the usual consequence of infection from the perspective of the human host is tissue damage. The extent of cellular damage caused by a given parasite relates to its essential niche's location, the parasite's metabolic requirements, and the number of organisms produced throughout the infection. The penchant of the parasite for killing the cell it invades or eroding the tissue it occupies while feeding on our cells results in measurable pathological consequences that translate directly into clinical signs and symptoms.

For example, when the malaria parasite exits from the red cell at the end of its division cycle, the rupture of the stroma releases toxic waste products (hemozoin) that elicit fever. *Entamoeba histolytica*, as its name implies, attaches to and then ingests living cells. It then digests them, using acid hydrolases to do

so, and in the process, induces bloody diarrhea (dysentery).

Infection with *Toxoplasma gondii* results in lymphadenopathy and fever due to the death of many host cells. When known, the molecular basis for these pathological effects will be discussed in detail for each parasite species. Much of this information is incomplete and is still under investigation.

Parasitic Helminths (worms)

Helminths belong to four phyla: Nematoda (roundworms), Platyhelminthes (flatworms), Acanthocephala (spiny-headed worms), and Nematophora (hairworms). Only worms belonging to the first three phyla are parasitic in humans. Both the Nematoda and Platyhelminthes have many free-living species as well. A general description of each significant group precedes each section. What follows is an overview of their biology related to their parasitic nature.

Mechanisms of Entry

Helminths have evolved multiple strategies for entering the host and establishing infection. Many nematode parasites produce eggs that are swallowed, initiating infection. Many species produce eggs that can live in the external environment for several years.

In many tropical countries, helminth eggs have been isolated from nearly all environments. They have even been recovered from paper currency. For other nematodes, infection is established when larval stages living in fecally contaminated soil enter the host by penetrating the unbroken skin, aided by hydrolytic enzymes. Sometimes, infection is strictly food-borne and occurs only when larvae are ingested in raw or undercooked meat. Many species of nematode are transmitted by arthropods, such as lymphatic filariasis

(mosquito), loiasis (deer fly), onchocerciasis (black fly), and Guinea worm (copepods).

Trematodes spend a portion of their life cycle in various snail intermediate hosts. After exiting the snail, the larval stage, known as a cercaria, typically attaches to a second intermediate host, such as a fish, a crab, or aquatic vegetation. For this reason, most trematode infections are food-borne. The exception is the Schistosomes, which cause a spectrum of illnesses. Due to the thinner epidermis, Schistosome cercariae can penetrate through the hair follicle, the invaginated epidermal layer that forms the hair shaft.

Cestodes are acquired via the oral route, regardless of the stage that causes the infection. Most adult tapeworm infections in humans result from ingesting inadequately cooked contaminated fish, beef, or pork. Two clinically significant juvenile tapeworm infections, cysticercosis, and echinococcosis, result from accidental ingestion of the eggs.

Niche Selection

Unlike protozoans, most species of parasitic helminths occupy more than a single niche in their human host during their life cycle. For example, although hookworms live as adults in the small intestine, to arrive there, the infective larvae frequently must first pass through the skin and lymphatics before spending time in the bloodstream and lungs. They then migrate up the respiratory tree and into the oral cavity, where they are swallowed, ending up in the gut. Similarly, *Ascaris* eggs hatch in the intestine before the emerging larval stage enters the portal circulation; the larvae enter the liver and lungs before re-entry into the gut. As adults, helminths have been recovered from almost every organ, including the liver, lungs, lymphatics, bloodstream, kidney, muscle, skin, subcutaneous tissues, and brain.

Many parasitic helminths live as sexually mature adults in the gastrointestinal (GI) tract. In many underdeveloped countries, it is common to find school-aged children who harbor three or four different species of helminths in their intestines, with each species occupying a different portion of the GI tract. Symptoms arising from heavy infection with a given helminth are typically associated with a particular region of the GI tract.

Reproduction

Nematode parasites in the GI tract produce eggs or larvae that exit the host with the fecal mass. Nematodes living in blood or lymphatic vessels have larvae that circulate in the bloodstream and must be ingested by the appropriate arthropod vector to exit the host.

The situation is different for cestodes, as each proglottid (segment) of the adult tapeworm is hermaphroditic. Because only one adult worm is usually present, the worm self-fertilizes adjacent segments. Adult tapeworms shed segments into the lumen of the small intestine, and they can exit the host under their own power. Other adult tapeworms produce segments that disintegrate, releasing their eggs into the fecal mass for export. Juvenile tapeworm infections produce no diagnostic stage that can be easily accessed. These infections are difficult to identify, requiring biopsy, imaging strategies, and confirmatory serological or molecular tests to speciate the infective juvenile worm.

Except for the schistosomes, the trematodes (flukes) are all hermaphroditic. Despite this all-in-one reproductive arrangement, cross-fertilization between two trematodes of the same species is common. Intestinal trematodes produce eggs that exit with the feces, for example, with the eggs of *Heterophyes heterophyes*. Eggs of the lung fluke, *Paragonimus westermani*, exit the host either

when they are coughed up in sputum or after they are swallowed, in which case they exit in the feces.

Some helminths have evolved elaborate adaptations to ensure their eggs leave the human host. For instance, schistosome eggs are deposited against the inside wall of a blood vessel. Their eggs have sharp spines and a battery of lytic enzymes that allow them to traverse the vessel endothelium and gut or bladder wall. The eggs traverse the serosal surface of the intestine or bladder (depending upon the species) before entering the muscularis layer and the lumen. Adult Schistosomes and *Paragonimus* can locate ectopic sites (e.g., the nervous system), producing eggs that remain at the site of infection, often resulting in serious pathological consequences for the host.

Mechanisms of Survival

Like the protozoa, the helminths occupy essential niches that most immunologists consider highly inhospitable. The selective pressures that led to their elaborate mechanisms for survival in these environments are still poorly understood. Adult Schistosomes live in the bloodstream, where one might expect a constant attack from the immune system's antibody molecules and leukocytes of various types. The worms thrive in that niche for up to twenty years.

The precise molecular basis by which this happens is not known, although several immune evasion and immunological masking mechanisms have been described. Essential for helminth survival is their repertoire of natural products elaborated and released into the host. Hookworms can freely ingest blood in the intestinal mucosa and submucosa because they produce peptides and eicosanoids that block host clotting, host platelet aggregation, and host inflammation. Many of these peptides have proven helpful as new potential

therapeutic agents for human coronary artery disease, stroke, and autoimmune disorders. *Trichuris trichiura* releases a pore-forming protein that promotes cell fusion around the anterior end of the organism, allowing it to become embedded in epithelial tunnels.

Indeed, the argument has been made that parasitic helminths are themselves equivalent to small biotechnology companies. Through research and development, millions of years of evolutionary selection have produced a wide array of active compounds (parapharmaceuticals), which we may eventually find helpful, as well.

Mechanisms of Pathogenesis

Helminths injure their human host both through mechanical and chemical mechanisms. Large helminths, such as *Ascaris lumbricoides*, can cause physical obstruction of the intestine or exert damage when they migrate into the biliary tree. As noted, helminths release peptides and eicosanoids that down-regulate host inflammatory processes. In some cases, helminths bias host immunity to produce Th-2-like responses that may make the host less likely to eliminate the parasite.

Immune regulation on the part of the parasite may also have consequences for the host regarding a wide variety of viral infections.

Some evidence supports the role of helminths as co-pathogens that promote susceptibility to HIV infection, AIDS, and SARS-CoV2.¹ In many cases, some of the most important, clinically relevant pathogenesis mechanisms are still unknown. Heavy infection with some intestinal nematodes (e.g., hookworm) is the primary cause of stunted growth during childhood and induces impaired cognitive behavior and intellectual development. We might suspect that parasite-induced malnutrition plays an essential role in this process, but the basis of these processes is unknown.

Host-mediated immunopathology accounts for a considerable measure of the damage during some helminth infections. This is particularly true for infection with the Schistosomes. However, current evidence suggests that in the case of infection with several filarial worm species, a bacterial endosymbiont, *Wolbachia*, is required for worm survival and, hence, the pathological consequences of the helminth infection. Brain parenchymal inflammation and seizures in cysticercosis are well documented.

The genomes of many of these important human pathogens are now available, so new approaches to the clinical management of patients suffering from them will surely emerge from the laboratory and find their way to the bedside.

References

1. Ademe, M.; Girma, F., The Influence of Helminth Immune Regulation on COVID-19 Clinical Outcomes: Is it Beneficial or Detrimental? *Infect Drug Resist* **2021**, *14*, 4421-4426.



Bailey K. Ashford, M.D. (1873–1934)

Ashford identified hookworm infection as the primary cause of “Puerto Rico anemia” that affected nearly 60% of the rural population. He instituted successful treatment and intervention programs (construction of latrines and public health education), which significantly reduced the death rate from anemia due to this parasite. In 1925, Ashford helped to establish a school of public health in the city of Puerta de Tierra.

IV. The Protozoa

Over 200,000 species of protozoa have been described so far, of which more than half are represented in the fossil record. The repertoire of known living species (approximately 65,000) includes more than 10,000 parasites that infect a broad spectrum of vertebrate and invertebrate hosts. All protozoans are eukaryotic single-cell organisms. Free-living species occupy many ecological niches, including marine trenches, rainforests, artesian and thermal springs, salt lakes, ice flows, glaciers, etc.

In contrast to most helminth parasites, parasitic protozoans replicate once inside their hosts, often resulting in hundreds of thousands of new organisms within just a few days of initial infection. This biological feature frequently has grave consequences.

Parasitic protozoans have played a significant role in the evolution of the human species, mainly due to their lethality or limiting where people can live by adversely affecting their livestock. These selection pressures continue to play out in many parts of the world. For example, malaria, African trypanosomiasis, and visceral leishmaniasis infect millions of people and are responsible for untold deaths and debilitating chronic illnesses. Many others cause less severe disease (e.g., chronic diarrhea), resulting in lost time at work and school and loss of time spent on recreational activities. This is due, in part, to the fact that some important species of parasitic protozoans are no longer susceptible to drugs that were once effective in limiting disease.

While the biology of parasitic protozoa varies widely from group to group, many share common features. They are all bound by a double-unit membrane. Nutrients may be actively transported, phagocytized, or moved into the cell by pinocytosis. Digestion

of particulate material is by lysosomal enzymes within the phagolysosome. Protozoans excrete wastes either by diffusion or by exocytosis. Mechanisms of motility take advantage of the presence of one of a variety of structures (e.g., cilia, flagella, pseudopod). All species of protozoa can divide asexually, usually by binary fission. Sometimes, the process is more complex and includes multiple nuclear divisions followed by cytokinesis. Those capable of sexual reproduction do so within the definitive host, forming a zygote.

Their cytoplasm may contain subcellular organelles, including Golgi apparatus, lysosomes, mitochondria, rough and smooth endoplasmic reticulum, and a wide variety of membrane-bound vesicles of specialized function (e.g., the hydrogenosome of *Trichomonas vaginalis*, and the glycosome of kinetoplastidae). Collectively, these cytoplasmic inclusions enable the organism to respire, digest food, generate energy, grow, and reproduce. Mitosomes are thought to have evolved from mitochondria, but are not involved in aerobic energy metabolism. Mitosomes are found in a few parasitic protozoa, for example, *Giardia lamblia* and *Cryptosporidium parvum*. Its function is linked to the partitioning of cytoplasmic components just before cell division.

Some species have evolved elaborate surface coats consisting of macromolecules derived either from the host or secreted by the parasite (for example, *Trypanosoma brucei*), that offer protection from host immune responses, thereby extending their life within a given individual.

The fields of immunoparasitology, parasite genomics, and parasite proteomics have also matured over the past several years. New understanding regarding the role(s) of cytokines and interleukins in the pathogenesis of disease has led to new clinical approaches

for several important protozoan diseases. In addition, recently discovered details of protective host immune mechanisms give hope for developing a new generation of drugs and perhaps even the first of many effective vaccines.

The following chapters are but a thumbnail sketch of some of the excitement generated in the field of protozoan parasitology. They are designed to present to the student and clinician valuable and practical information specific to diagnosing, treating, and managing infections caused by these pathogens.



Joseph Bancroft, M.D. (1836–1894)

Bancroft's life-long passion for medicine was augmented by his interest in edible plants. He conducted research aimed at improving disease resistance for some important commercial crops (e.g., wheat, sugar cane, and bananas). Bancroft also worked on leprosy and was the first to describe the adult worm of *Wuchereria bancrofti*, which bears his name and that of Wucherer.

While Patrick Manson had observed that mosquitos were intermediate hosts of *Wuchereria bancrofti*, in 1899, only 6 years after Theobald Smith and Frederick Kilbourne were the first to demonstrate that ticks could transmit disease, Bancroft was able to demonstrate that the filarial parasite, *Wuchereria bancrofti* could be transmitted back to humans from the bite of a mosquito.

1. *Giardia lamblia* (also known as *G. duodenalis* or *G. intestinalis*) (Stiles 1915)

Pronunciation: |jē-är'dē-ə|\lām'blē-ə|

Introduction

Giardia lamblia (|jee-ARE-dee-ah|\lam-BLEE-ah|), also known as *G. duodenalis* or *G. intestinalis*) is a flagellated protozoan that lacks a mitochondrion.¹ It is aerotolerant but respire as an anaerobe and lives in the small intestine. Other protozoa sharing this metabolic strategy include *Entamoeba histolytica* and *Trichomonas vaginalis*. There are currently nine validated *Giardia* spp. and eight established genotypes known as assemblages A to H that infect mammals with groups A and B infecting humans.^{2,3} Genotypes A and B have been isolated from humans and other mammals.⁴ *G. lamblia* produces an environmentally resistant cyst stage.⁵

Infection is via the fecal-oral route, typically through exposure to contaminated drinking water.⁶ *G. lamblia* has a worldwide distribution and is endemic in many regions.⁷ Giardiasis frequently occurs in children (especially those attending daycare centers), travelers, immunocompromised individuals (including HIV-infected individuals), and patients with genetic disorders such as cystic fibrosis.⁸⁻¹³ *G. lamblia* is a common infection in humans and domestic animals in the United States.¹⁴ Many infected individuals likely remain undiagnosed, harboring *G. lamblia* without apparent symptoms.¹⁵

Water-dwelling animals such as beavers can be infected and contaminate public drinking water. This is why infection with *G. lamblia* is known in many parts of the United States as “beaver fever.”^{16,17} *Giardia* is the subject of much intensive research, including the complete sequencing of its genome.¹⁸ A survey of

its genome has revealed the presence of genes for meiosis, and population genetics suggest some form of genetic exchange exists, possibly during encystation or excystation. Yet, a sexual stage for this protozoan has not been established.^{19,20} Although an excellent review of the biology of *G. lamblia* was published by Adam in 2001,²¹ much has been added to the field since this comprehensive report.²²

Historical Information

Antonie van Leeuwenhoek (|LAY-vun-HOOK|, |lā-vən-ˌhük|), the famous Dutch microscopist, in a letter written to Robert Hooke (|HOOK|, |hük|) in 1681, described in detail the living trophozoite stage of *Giardia*, which he observed in a sample of his stool: “. . . animalcules a-moving very prettily. Their bodies were somewhat longer than broad, and their belly, which was flatlike, furnisht with sundry little paws. . . yet for all that they made but slow progress.”²³

In 1859, Vilem Lambl described the main morphological features of the trophozoite stage that he obtained from the stools of various pediatric patients in Prague.²⁴ His elegant scientific drawings remain impressive, even in today’s sophisticated, technologically advanced light microscopy world. In 1921, C.E. Simon completed the description of its morphology.²⁵



Figure 1.1. Trophozoite of *Giardia lamblia*. 15 μm.

Giardia lamblia

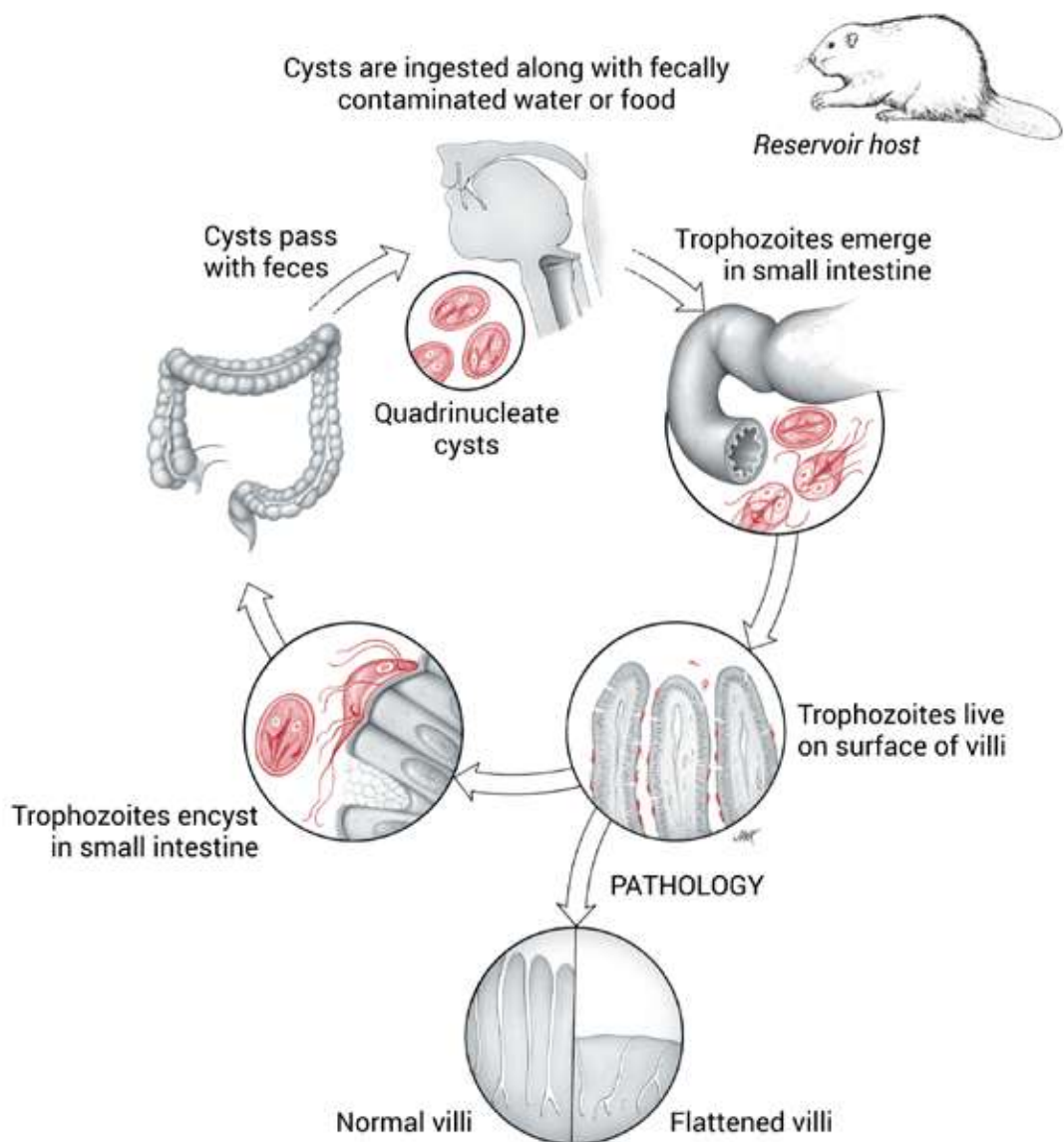




Figure 1.2. Cyst of *G. lamblia*. Two nuclei can be seen. 13 μm .

Life Cycle

G. lamblia exists in two forms: the trophozoite (Fig. 1.1) and the cyst (Fig. 1.2). The trophozoite is pear-shaped and motile, measuring 10–20 μm by 7–10 μm . It possesses eight flagella and is bi-nucleate. Both nuclei are transcriptionally active.²⁶ In addition, it contains two rigid structures, called median bodies, which are now known to be part of the complex and unique cytoskeleton.²⁷ *G. lamblia* has no mitochondria, peroxisomes, hydrogenosomes, or related subcellular organelles that might be associated with energy metabolism. Still, it does appear to use a homolog of the mitochondrial-like glycerol-3-phosphate dehydrogenase (GPDH) involved in glycolysis.²⁸ Some parasite strains carry double-stranded RNA viruses, known as *Giardiaviruses*, whose impact on virulence is still being explored.^{29,30} These viruses have facilitated the expression of foreign genes in *Giardia*, serving as shuttle vectors.³¹

G. lamblia's anterior ventral region has a disc-like, specialized organelle that it uses to attach to the surface of epithelial cells. The integrity of the disk is maintained by tubulin and giardins.³² The latter are members of class III, which has low affinity and calcium-binding annexins.³³ The disc structure has been inves-

tigated using cryoelectron microscopy.³⁴

Infection begins with ingesting the quadri-nucleate cyst, which must then excyst in response to a complex sequence of environmental cues the parasite receives.³⁵ Ingesting the trophozoite stage does not result in infection. As the cyst passes through the stomach and into the small intestine, it is sequentially exposed to hydrochloric acid and pancreatic enzymes.³⁶ Excystation may be inhibited by ethanol and isopropanol-containing hand sanitizers.³⁷

Each cyst produces two bi-nucleate trophozoites that attach to epithelial cells using the ventral disk (Fig. 1.3). *G. lamblia* binding appears to use an epsinR homolog (Glepsin) to bind both phosphatidylinositol (3,4,5)-triphosphate phospholipids without canonical domains for interaction with clathrin coat components.³⁸ Once attached to epithelial cells, the bi-nucleate trophozoites grow and divide by binary fission. Cysts are unable to replicate.

G. lamblia can be grown *in vitro* (Fig. 1.4). The complete nutritional needs of the trophozoite have yet to be entirely determined, but some of its biochemical energy pathways are known.^{39,40} Glucose and arginine appear to be their major sources of energy, and they may access a portion of their need through

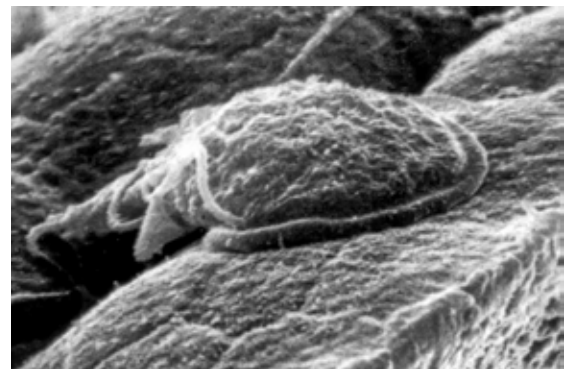


Figure 1.3. Scanning EM of a trophozoite of *G. muris* on epithelium of mouse small intestine. Courtesy R. Owen.



Figure 1.4. Trophozoites of *G. lamblia* in culture. Courtesy D. Lindmark.

the breakdown of mucus.^{41, 42} *Giardia* cannot synthesize nucleic acid bases *de novo* and consequently employs salvage pathways.⁴³ Lipids are absorbed directly, likely facilitated by bile and bile salts and perhaps by endocytosis of lipoproteins.^{44, 45}

G. lamblia is not considered an invasive or tissue parasite. Its ability to adhere closely to the columnar cells at the level of the microvilli and its penchant for secreting proteins at the site stimulates antibody production and, eventually, protective immunity.⁴⁶ To exit the host and survive, trophozoites must enter the large intestine. Bile salts seem to be involved in triggering this process.^{5, 47, 48} Encystation *in vitro* is achieved by exposing the trophozoite stage to bile and elevated pH, possibly

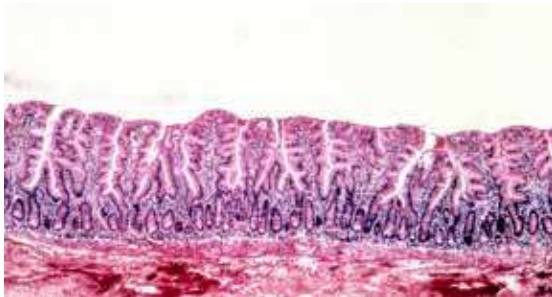


Figure 1.5. Flattened, fused villi of small intestine from a patient suffering from malabsorption syndrome due to *G. lamblia*.

stimulating the parasite to sequester cholesterol.⁴⁹ Trophozoites take up and release conjugated bile salts.^{48, 50} These conditions may be necessary for its survival in its essential niche (Fig. 1.3). A transglutaminase may also be required for encystment.⁵¹

Encysted parasites can endure for long periods outside the host if they remain moist and the temperature is not elevated.⁵² Both cysts and trophozoites pass out of the bowel with the fecal mass, but only the cyst stage survives. Cysts can withstand exposure to mild chemical treatments, such as chlorinated water, for short periods at low temperatures.⁵³ Freezing, boiling, UV exposure, or desiccation can destroy cysts.⁵⁴ Production of cysts occurs throughout the infection, but the number produced each day varies greatly, depending upon a wide variety of conditions, including the development of acquired protective immunity.⁵⁵ Protective immunity appears to be directed against surface and secreted antigens.^{56, 57}

Cellular and Molecular Pathogenesis

The dominant pathological consequences of chronic infection are steatorrhea and malabsorption with flattening of the villi (Fig. 1.5), often accompanied by profound weight loss.^{58, 59} Although numerous related *Giardia* species can be manipulated *in vivo* and *in vitro*, surprisingly, little is known regarding their biological effect(s) on the physiology and biochemistry of the small intestine.²¹ The diarrhea generated by *G. lamblia* appears partly due to augmentation of peristalsis and alteration of host cell tight junctions.^{60, 61}

Infection with *G. lamblia* induces numerous cellular and humoral responses, some of which are protective.⁶²⁻⁶⁴ Secretory IgA is essential in controlling the infection.⁶⁵ Physiological changes, such as flattening of the villi,

experienced during symptomatic infection could relate to these host-based responses. They might be induced by mechanisms related to allergies, such as those observed in gluten-sensitive individuals.^{63, 64}

Antigenic variation of surface components of the trophozoite is typical in the early phase of infection and most likely aids the parasite in avoiding elimination by humoral responses (e.g., IgA antibodies) directed at trophozoite surface proteins.⁶⁶ Switching of cysteine-rich variant surface proteins also occurs when the parasite is about to excyst, assisting the parasite in evading immune elimination.^{56, 65, 67, 68} Severe combined immune deficiency mice do not induce variant surface protein switching, suggesting that B cell-mediated host responses control this process. Shuttle viral systems for transfecting *G. lamblia* have been developed.⁶⁹ Thus, genetic manipulation is now possible, which may lead to a more complete understanding of the molecular events governing pathogenesis.

Human breast milk is protective because it contains antibodies of the IgA class.⁷⁰ Non-specific defenses, such as lactoferrin or products of lipid hydrolysis of the milk in the normal digestive tract, may also play a role, as each is toxic to *Giardia*.⁷¹⁻⁷³ In response to infection, NO is released luminally by intestinal epithelial cells, which inhibits parasite growth and differentiation. However, *Giardia* might be able to disarm this potential defense mechanism by competitively consuming the arginine needed by the host cells for NO synthesis.⁷⁴ In summary, the duration and severity of infection depend upon both immune and nonimmune host defenses and the parasite's ability to evade them.



Figure 1.6a. *G. lamblia* trophozoite in stool sample.

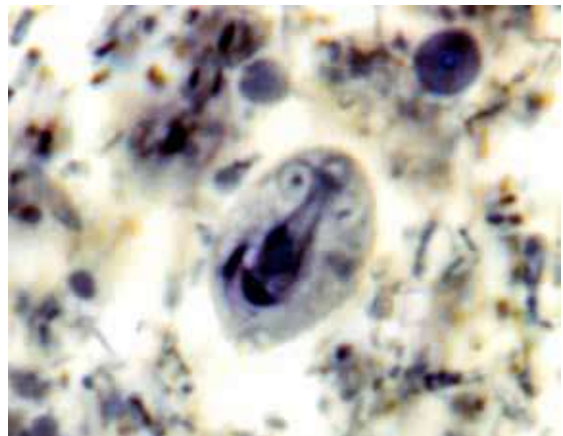


Figure 1.6b. *G. lamblia* cyst in stool sample.

Clinical Disease

It is estimated that many who encounter *G. lamblia* and become infected fail to progress to ill health.^{75, 76} Infected individuals may remain asymptomatic for long periods, becoming chronic carriers referred to as *cyst passers*. Of those who go on to develop disease, the most prominent symptom is protracted diarrhea.^{42, 77} The acute diarrhea of giardiasis is foul-smelling, with flatulence, nausea, weight loss, abdomi-

nal cramps, and bloating.⁷⁸ A minority of patients may describe systemic symptoms such as fever.⁷⁸ Untreated, this type of diarrhea may last weeks or months, although it usually varies in intensity. Affected children often fail to thrive.⁷⁹ Chronic infections are characterized by steatorrhea and malabsorption syndrome and are associated with rapid, substantial weight loss, general debility, and consequent fatigue.⁴² Also, some people may complain of epigastric discomfort, anorexia, and even pain.

Certain patient groups are at greater risk of acquiring giardiasis and developing chronic infection. Patients suffering from immunocompromising conditions (e.g., hypogammaglobulinemia, patients unable to secrete IgA, HIV/AIDS, or undertaking cancer chemotherapies), cystic fibrosis and children with underlying malnutrition can experience a protracted disease with more severe symptoms.^{13, 80-82}

Diagnosis (see Clinical Appendix)

The diagnosis of giardiasis has changed dramatically with the introduction of newer diagnostic techniques. Historically, definitive diagnosis depended upon direct, microscopic observation of trophozoites (Fig. 1.6a) or cysts (Fig. 1.6b) in concentrated stained stool.⁸³ Antigen-capture ELISA was introduced for several reasons, including the inherent challenges of obtaining such specimens and the limited number of skilled laboratory personnel needed to examine them.⁸⁴ These stool antigen detection assays, particularly the direct fluorescent antibody test (DFA), have greater sensitivity than stool microscopy, faster turnaround time, and only require a single stool collection.^{85, 86} The string test involved swallowing a gelatin capsule attached to the end of a long string and is now relegated to a place in history, as newer, better-

tolerated diagnostic techniques are becoming available.⁸⁷ NAATs are now available and are revolutionizing the diagnosis of infectious diarrhea. The BioFire, FilmArray, and the Luminex xTAG Gastrointestinal Pathogen Panels are among the commercially available NAAT tests that can detect, with high sensitivity and specificity, a broad number of viral, bacterial, and protozoan pathogens.⁸⁸⁻⁹⁰

Treatment (see Clinical Appendix)

It is recommended that all symptomatic patients infected with *Giardia* be treated with antimicrobial therapy, as this has been shown to relieve symptoms with minimal side effects.⁹¹ Nitroimidazoles, metronidazole, and tinidazole are the primary drugs used for treatment.^{91, 92} Metronidazole, an inexpensive option, is usually given at a dose of 250 mg by mouth three times per day for 5–7 days for adults, while tinidazole can be given as a single oral dose of 2 grams with high efficacy. Another preferred drug option is nitazoxanide 500 mg orally twice daily for three days.^{93, 94} Recommended doses of these medications are weight-based in children. Alternative antimicrobials include paromomycin (in pregnancy), furazolidone, quinacrine, and alben-dazole.⁹¹

Recurrence or persistence of symptoms should be evaluated carefully, as persisting malabsorption and lactose intolerance can last for weeks to months following infection.⁹⁵ Before retreatment, it is recommended that one re-evaluate the patient and confirm the presence of infection.⁹⁶ Because resistant strains of *Giardia* are increasingly prevalent, many patients will need to be retreated, given a different class of antimicrobial therapy, or a longer course of the original agent.⁹⁷⁻¹⁰⁰ In some refractory cases, combination antimicrobial therapy may be necessary.⁹³

Prevention and Control

G. lamblia is primarily a water-borne infection, although food handlers and infected children in daycare centers no doubt play essential roles in transmission.^{6,101} Sexual transmission has also been increasingly described.¹⁰² Prevention strategies include proper disposal

of human waste, filtration of drinking water supplies, maintenance of buffer zones around watersheds when filtration is not practiced (e.g., in New York City), and maintaining the highest hygiene standards in daycare centers and mental institutions. A murine model for a protective *Giardia* vaccine exists, and work continues on vaccine development.^{103, 104}

References

1. Roger, A. J., et al., A mitochondrial-like chaperonin 60 gene in *Giardia lamblia*: evidence that diplomonads once harbored an endosymbiont related to the progenitor of mitochondria. *Proceedings of the National Academy of Sciences of the United States of America* **1998**, *95* (1), 229-34.
2. Vanni, I., et al., Detection of *Giardia duodenalis* assemblages A and B in human feces by simple, assemblage-specific PCR assays. *PLoS Negl Trop Dis* **2012**, *6* (8), e1776.
3. Ryan, U. M., et al., Taxonomy and molecular epidemiology of *Cryptosporidium* and *Giardia* - a 50 year perspective (1971-2021). *Int J Parasitol* **2021**, *51* (13-14), 1099-1119.
4. Zajackowski, P., et al., The controversies surrounding *Giardia intestinalis* assemblages A and B. *Curr Res Parasitol Vector Borne Dis* **2021**, *1*, 100055.
5. Faso, C., et al., The proteome landscape of *Giardia lamblia* encystation. *PloS one* **2013**, *8* (12), e83207.
6. Levy, D. A., et al., Surveillance for waterborne-disease outbreaks--United States, 1995-1996. *MMWR. CDC surveillance summaries : Morbidity and mortality weekly report. CDC surveillance summaries / Centers for Disease Control* **1998**, *47* (5), 1-34.
7. Feng, Y.; Xiao, L., Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. *Clin Microbiol Rev* **2011**, *24* (1), 110-40.
8. Sagi, E. F., et al., *Giardia lamblia*: prevalence, influence on growth, and symptomatology in healthy nursery children. *Isr Sci* **1983**, *19*, 815-817.
9. Pickering, L. K., et al., Occurrence of *Giardia lamblia* in children in day care centers. *The Journal of pediatrics* **1984**, *104* (4), 522-6.
10. Daszak, P., *Giardia*, HIV, and nature's horrifying beauty. *Ecohealth* **2014**, *11* (2), 277-8.
11. Cimino, A.; Ali, S. Z., *Giardia intestinalis* on anal PAP of an HIV-positive male. *Diagn Cytopathol* **2010**, *38* (11), 814-5.
12. Jelinek, T.; Loscher, T., Epidemiology of giardiasis in German travelers. *J Travel Med* **2000**, *7* (2), 70-3.
13. Roberts, D. M., et al., Prevalence of giardiasis in patients with cystic fibrosis. *The Journal of pediatrics* **1988**, *112* (4), 555-9.
14. Mohamed, A. S., et al., Temporal patterns of human and canine *Giardia* infection in the United States: 2003-2009. *Prev Vet Med* **2014**, *113* (2), 249-56.
15. Nash, T. E., et al., Experimental human infections with *Giardia lamblia*. *J Infect Dis* **1987**, *156* (6), 974-84.
16. Carlson, D. W.; Finger, D. R., Beaver fever arthritis. *J Clin Rheumatol* **2004**, *10* (2), 86-8.
17. Taverne, J., Beaver fever and pinworm neuroses on the Net. *Parasitol Today* **1999**, *15* (9), 363-4.
18. Best, A. A., et al., Evolution of eukaryotic transcription: insights from the genome of *Giardia lamblia*. *Genome research* **2004**, *14* (8), 1537-47.
19. Ramesh, M. A., et al., A phylogenomic inventory of meiotic genes; evidence for sex in *Giardia* and an early eukaryotic origin of meiosis. *Current biology : CB* **2005**, *15* (2), 185-91.
20. Weedall, G. D.; Hall, N., Sexual reproduction and genetic exchange in parasitic protists. *Parasitology* **2015**, *142 Suppl 1*, S120-7.

21. Adam, R. D., Biology of *Giardia lamblia*. *Clinical microbiology reviews* **2001**, 14 (3), 447-75.
22. Leung, A. K. C., et al., Giardiasis: An Overview. *Recent Pat Inflamm Allergy Drug Discov* **2019**, 13 (2), 134-143.
23. Leenwenhoek, A.; Dobell, C., Van Cited by In Antony van Leeuwen-hoek and His "Little Animals". *Publications New Dover P* **1960**, 224.
24. Lambi, V. D. F., Mikroskopische Untersuchungen der Darm-Excrete. *Beitrag zur Pathologie des Darms und zur Diagnostik am Krankenbette Vierteljahrschrift für die Praktische Heilkunde Med Fac Prague* **1859**, 1, 1-58.
25. Simon, C. E.; J., *Giardia enterica*, a parasitic intestinal flagellate of man. *Am* **1921**, 1, 440-491.
26. Kabnick, K. S.; Peattie, D. A., *In situ* analyses reveal that the two nuclei of *Giardia lamblia* are equivalent. *Journal of cell science* **1990**, 95 (Pt 3), 353-60.
27. Piva, B.; Benchimol, M., The median body of *Giardia lamblia*: an ultrastructural study. *Biol Cell* **2004**, 96 (9), 735-46.
28. Lalle, M., et al., The FAD-dependent glycerol-3-phosphate dehydrogenase of *Giardia duodenalis*: an unconventional enzyme that interacts with the g14-3-3 and it is a target of the antitumoral compound NBDHEX. *Front Microbiol* **2015**, 6, 544.
29. Tai, J. H., et al., Separation and characterization of two related *Giardiaviruses* in the parasitic protozoan *Giardia lamblia*. *Virology* **1996**, 216 (1), 124-32.
30. Janssen, M. E., et al., Three-dimensional structure of a protozoal double-stranded RNA virus that infects the enteric pathogen *Giardia lamblia*. *J Virol* **2015**, 89 (2), 1182-94.
31. Liu, Q., et al., *Giardia lamblia*: stable expression of green fluorescent protein mediated by giardiavirus. *Experimental parasitology* **2005**, 109 (3), 181-7.
32. Aggarwal, A., et al., Characterization of a 29.4-kilodalton structural protein of *Giardia lamblia* and localization to the ventral disk. *Infection and immunity* **1989**, 57 (4), 1305-10.
33. Bauer, B., et al., Functional identification of alpha 1-giardin as an annexin of *Giardia lamblia*. *FEMS microbiology letters* **1999**, 173 (1), 147-53.
34. Brown, J. R., et al., A detailed look at the cytoskeletal architecture of the *Giardia lamblia* ventral disc. *J Struct Biol* **2016**, 194 (1), 38-48.
35. Hetsko, M. L., et al., Cellular and transcriptional changes during excystation of *Giardia lamblia* *in vitro*. *Experimental parasitology* **1998**, 88 (3), 172-83.
36. Lauwaet, T., et al., Encystation of *Giardia lamblia*: a model for other parasites. *Curr Opin Microbiol* **2007**, 10 (6), 554-9.
37. Chatterjee, A., et al., Ethanol and isopropanol in concentrations present in hand sanitizers sharply reduce excystation of *Giardia* and *Entamoeba* and eliminate oral infectivity of *Giardia* cysts in gerbils. *Antimicrob Agents Chemother* **2015**, 59 (11), 6749-54.
38. Ebnetter, J. A.; Hehl, A. B., The single epsin homolog in *Giardia lamblia* localizes to the ventral disk of trophozoites and is not associated with clathrin membrane coats. *Mol Biochem Parasitol* **2014**, 197 (1-2), 24-7.
39. Jarroll, E. L., et al., Biochemistry and metabolism of *Giardia*. *The Journal of protozoology* **1989**, 36 (2), 190-7.
40. Coombs, G. H., et al., Energy Metabolism in Anaerobic Protozoa. 1995; p 109-131.
41. Edwards, M. R., et al., Arginine metabolism during culture of *Giardia intestinalis*. *Mol Biochem Parasitol* **1992**, 103, 1-2.
42. Farthing, M. J. G., et al., *Giardia lamblia*. 1998; p 2399-2406.
43. Wang, C. C.; Aldritt, S., Purine salvage networks in *Giardia lamblia*. *The Journal of experimental medicine* **1983**, 158 (5), 1703-12.
44. Farthing, M. J., et al., Effects of bile and bile salts on growth and membrane lipid uptake by *Giardia lamblia*. Possible implications for pathogenesis of intestinal disease. *The Journal of clinical investigation* **1985**, 76 (5), 1727-32.
45. Lujan, H. D., et al., Lipid requirements and lipid uptake by *Giardia lamblia* trophozoites in culture. *The Journal of eukaryotic microbiology* **1996**, 43 (3), 237-42.
46. Velazquez, C., et al., *Giardia lamblia* infection induces different secretory and systemic antibody responses in mice. *Parasite Immunol* **2005**, 27 (9), 351-6.

47. Lujan, H. D., et al., Mechanisms of *Giardia lamblia* differentiation into cysts. *Microbio Mol Biol Rev* **1997**, *61* (3), 294-304.
48. Halliday, C. E., et al., Characterization of bile salt uptake by *Giardia lamblia*. *Int J Parasitol* **1995**, *25* (9), 1089-97.
49. Lujan, H. D., et al., Cholesterol starvation induces differentiation of the intestinal parasite *Giardia lamblia*. *Proc Natl Acad* **1996**, *93*, 7628-33.
50. Halliday, C. E., et al., *Giardia*-bile salt interactions *in vitro* and *in vivo*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1988**, *82* (3), 428-32.
51. Davids, B. J., et al., Dependence of *Giardia lamblia* encystation on novel transglutaminase activity. *Molecular and biochemical parasitology* **2004**, *136* (2), 173-80.
52. Alum, A., et al., Impact of environmental conditions on the survival of *Cryptosporidium* and *Giardia* on environmental surfaces. *Interdiscip Perspect Infect Dis* **2014**, *2014*, 210385.
53. Jarroll, E. L., et al., Effect of chlorine on *Giardia lamblia* cyst viability. *Appl Environ Microbiol* **1981**, *41* (2), 483-7.
54. Einarsson, E., et al., UV irradiation responses in *Giardia intestinalis*. *Exp Parasitol* **2015**, *154*, 25-32.
55. Farthing, M. J.; Goka, A. J., Immunology of giardiasis. *Bailliere's clinical gastroenterology* **1987**, *1* (3), 589-603.
56. Nash, T. E., Antigenic variation in *Giardia lamblia* and the host's immune response. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **1997**, *352* (1359), 1369-75.
57. Kaur, H., et al., Immune effector responses to an excretory-secretory product of *Giardia lamblia*. *FEMS immunology and medical microbiology* **1999**, *23* (2), 93-105.
58. Carroccio, A., et al., Secondary impairment of pancreatic function as a cause of severe malabsorption in intestinal giardiasis: a case report. *Am J Trop Med Hyg* **1997**, *56* (6), 599-602.
59. Gottstein, B., et al., Human cellular immune response to *Giardia lamblia*. *Infection* **1991**, *19* (6), 421-6.
60. Buret, A. G., et al., *Giardia lamblia* disrupts tight junctional ZO-1 and increases permeability in non-transformed human small intestinal epithelial monolayers: effects of epidermal growth factor. *Parasitology* **2002**, *125* (Pt 1), 11-9.
61. Troeger, H., et al., Effect of chronic *Giardia lamblia* infection on epithelial transport and barrier function in human duodenum. *Gut* **2007**, *56* (3), 328-35.
62. Rosales-Borjas, D. M., et al., Secretory immune response to membrane antigens during *Giardia lamblia* infection in humans. *Infection and immunity* **1998**, *66* (2), 756-9.
63. Di Prisco, M. C., et al., Association between giardiasis and allergy. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology* **1998**, *81* (3), 261-5.
64. Doe, W. F., An overview of intestinal immunity and malabsorption. *The American journal of medicine* **1979**, *67* (6), 1077-84.
65. Eckmann, L., Mucosal defences against *Giardia*. *Parasite immunology* **2003**, *25* (5), 259-70.
66. Heyworth, M. F., Immunology of *Giardia* and *Cryptosporidium* infections. *J Infect Dis* **1992**, *166* (3), 465-72.
67. Nash, T. E., Surface antigenic variation in *Giardia lamblia*. *Molecular microbiology* **2002**, *45* (3), 585-90.
68. Svard, S. G., et al., Differentiation-associated surface antigen variation in the ancient eukaryote *Giardia lamblia*. *Molecular Microbiology* **1998**, *30* (5), 979-89.
69. Singer, S. M., et al., Episomal and integrated maintenance of foreign DNA in *Giardia lamblia*. *Molecular and biochemical parasitology* **1998**, *92* (1), 59-69.
70. Nayak, N., et al., Specific secretory IgA in the milk of *Giardia lamblia*-infected and uninfected women. *J Infect Dis* **1987**, *155* (4), 724-7.
71. Gillin, F. D., et al., Cholate-dependent killing of *Giardia lamblia* by human milk. *Infection and immunity* **1985**, *47* (3), 619-22.
72. Hernell, O., et al., Killing of *Giardia lamblia* by human milk lipases: an effect mediated by lipolysis of milk lipids. *J Infect Dis* **1986**, *153* (4), 715-20.

73. Reiner, D. S., et al., Human milk kills *Giardia lamblia* by generating toxic lipolytic products. *J Infect Dis* **1986**, 154 (5), 825-32.
74. Eckmann, L., et al., Nitric oxide production by human intestinal epithelial cells and competition for arginine as potential determinants of host defense against the lumen-dwelling pathogen *Giardia lamblia*. *Journal of immunology (Baltimore, Md. : 1950)* **2000**, 164 (3), 1478-87.
75. Ali, S. A.; Hill, D. R., *Giardia intestinalis*. *Current opinion in infectious diseases* **2003**, 16 (5), 453-60.
76. Lopez, C. E., et al., Waterborne giardiasis: a communitywide outbreak of disease and a high rate of asymptomatic infection. *Am J Epidemiol* **1980**, 112 (4), 495-507.
77. Reinthaler, F. F., et al., Diarrhea in returning Austrian tourists: epidemiology, etiology, and cost-analyses. *Journal of travel medicine* **1998**, 5 (2), 65-72.
78. Hopkins, R. S.; Juranek, D. D., Acute giardiasis: an improved clinical case definition for epidemiologic studies. *Am J Epidemiol* **1991**, 133 (4), 402-7.
79. Craft, J. C., *Giardia* and giardiasis in childhood. *Pediatric infectious disease* **1982**, 1 (3), 196-211.
80. Moolasart, P., *Giardia lamblia* in AIDS patients with diarrhea. *J Med Assoc Thai* **1999**, 82 (7), 654-9.
81. Bhajjee, F., et al., Human immunodeficiency virus-associated gastrointestinal disease: common endoscopic biopsy diagnoses. *Patholog Res Int* **2011**, 2011, 247923.
82. Sullivan, P. B., et al., *Helicobacter pylori* in Gambian children with chronic diarrhoea and malnutrition. *Archives of disease in childhood* **1990**, 65 (2), 189-91.
83. Kabani, A., et al., Practice guidelines for ordering stool ova and parasite testing in a pediatric population. The Alberta Children's Hospital. *American journal of clinical pathology* **1995**, 104 (3), 272-8.
84. Boone, J. H., et al., TechLab and alexon *Giardia* enzyme-linked immunosorbent assay kits detect cyst wall protein 1. *Journal of clinical microbiology* **1999**, 37 (3), 611-4.
85. Weitzel, T., et al., Evaluation of seven commercial antigen detection tests for *Giardia* and *Cryptosporidium* in stool samples. *Clin Microbiol Infect* **2006**, 12 (7), 656-9.
86. Jahan, N., et al., A Comparison of Microscopy and Enzyme Linked Immunosorbent Assay for Diagnosis of *Giardia lamblia* in Human Faecal Specimens. *J Clin Diagn Res* **2014**, 8 (11), DC04-6.
87. Jones, J. E., String test for diagnosing giardiasis. *American family physician* **1986**, 34 (2), 123-6.
88. Buss, S. N., et al., Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. *J Clin Microbiol* **2015**, 53 (3), 915-25.
89. Mengelle, C., et al., Simultaneous detection of gastrointestinal pathogens with a multiplex Luminex-based molecular assay in stool samples from diarrhoeic patients. *Clin Microbiol Infect* **2013**, 19 (10), E458-65.
90. Claas, E. C., et al., Performance of the xTAG(R) gastrointestinal pathogen panel, a multiplex molecular assay for simultaneous detection of bacterial, viral, and parasitic causes of infectious gastroenteritis. *J Microbiol Biotechnol* **2013**, 23 (7), 1041-5.
91. Granados, C. E., et al., Drugs for treating giardiasis. *Cochrane Database Syst Rev* **2012**, 12, CD007787.
92. Freeman, C. D., et al., Metronidazole. A therapeutic review and update. *Drugs* **1997**, 54 (5), 679-708.
93. Lopez-Velez, R., et al., Short course combination therapy for giardiasis after nitroimidazole failure. *Am J Trop Med Hyg* **2010**, 83 (1), 171-3.
94. Martinez-Rosas, V., et al., Nitazoxanide Inhibits the Bifunctional Enzyme GIG6PD::6PGL of *Giardia lamblia*: Biochemical and In Silico Characterization of a New Druggable Target. *Int J Mol Sci* **2023**, 24 (14).
95. Hanevik, K., et al., Development of functional gastrointestinal disorders after *Giardia lamblia* infection. *BMC Gastroenterol* **2009**, 9, 27.
96. Gardner, T. B.; Hill, D. R., Treatment of giardiasis. *Clin Microbiol Rev* **2001**, 14 (1), 114-28.
97. Abboud, P., et al., Successful treatment of metronidazole- and albendazole-resistant giardiasis

- with nitazoxanide in a patient with acquired immunodeficiency syndrome. *Clin Inf Dis* **2001**, 32 (12), 1792-4.
98. Fox, L. M.; Saravolatz, L. D., Nitazoxanide: a new thiazolide antiparasitic agent. *Clin Inf Dis* **2005**, 40 (8), 1173-80.
 99. Yereli, K., et al., Albendazole as an alternative therapeutic agent for childhood giardiasis in Turkey. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **2004**, 10 (6), 527-9.
 100. Miyamoto, Y.; Eckmann, L., Drug Development Against the Major Diarrhea-Causing Parasites of the Small Intestine, *Cryptosporidium* and *Giardia*. *Front Microbiol* **2015**, 6, 1208.
 101. Steiner, T. S., et al., Protozoal agents: what are the dangers for the public water supply? *Annual review of medicine* **1997**, 48, 329-40.
 102. Escobedo, A. A., et al., Sexual transmission of giardiasis: a neglected route of spread? *Acta Trop* **2014**, 132, 106-11.
 103. Jenikova, G., et al., Alpha1-giardin based live heterologous vaccine protects against *Giardia lamblia* infection in a murine model. *Vaccine* **2011**, 29 (51), 9529-37.
 104. Serradell, M. C., et al., Vaccination of domestic animals with a novel oral vaccine prevents *Giardia* infections, alleviates signs of giardiasis and reduces transmission to humans. *NPJ Vaccines* **2016**, 1, 16018.

2. Introduction to the *Leishmania* species

Pronunciation: \lēsh-'ma-nē-ə\

The genus *Leishmania* (\LEESH-ma-NEE-ah\) comprises a genetically diverse group of vector-borne hemoflagellate parasites.^{1, 2} *Leishmania* spp. are transmitted by the bite of sand flies (Fig. 2.1). There are two genera of sand flies: *Phlebotomus* spp., vectors of Old World Leishmaniasis, and *Lutzomyia* spp. transmitting leishmaniasis throughout the Western Hemisphere.³ *Psychodopygus* spp. and *Psathyromyia* spp. are newly identified vectors of *Leishmania* spp. reported in Brazil.^{4, 5}

Leishmania spp. are primarily zoonotic, infecting various vertebrates throughout the tropical and subtropical world.^{6, 7} All *Leishmania* spp. possess a well-characterized kinetoplast, a specialized organelle unique to the class kinetoplastidae.⁸ They live as obligate intracellular parasites within macrophages and other phagocytic cells of the reticuloendothelial system. Infection of multiple cell types has been demonstrated, including tissue-resident macrophages, dendritic cells, lymph node fibroblasts, and neutrophils.⁹ Species of *Leishmania* share many similarities in genetics, mode of transmission, biochemistry, molecular biology, immunobiology, and drug susceptibility. Divergence is greatest between the cutaneous and visceralizing species of *Leishmania*.



Figure 2.1. Sand fly taking a blood meal.

Leishmania spp. are known to cause several different disease manifestations, including visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), diffuse cutaneous leishmaniasis (DCL), leishmaniasis recidivans (LR), disseminated cutaneous leishmaniasis (disseminated CL), and diffuse cutaneous leishmaniasis (diffuse CL).¹⁰⁻¹² Disseminated CL: maculopapular lesions identified in two or more anatomical sites ranging from 10-300 in number, while multiple nonulcerative nodules characterize diffuse CL.^{11, 12} The exact number of humans suffering from leishmaniasis is unknown. The Centers for Disease Control and Prevention estimates suggest that the number of cases of VL may have decreased to less than 100,000 cases per year from over 400,000 while there still may be as many as 0.7–1.2 million cases of CL each year.¹³ According to estimates by the WHO and CDC, hundreds of millions of people live within an area of transmission. Leishmaniasis occurs in countries in Southern Europe, Africa, Asia, South Asia, South America, Central America, and even parts of the United States.^{14, 15} The subgenus *Leishmania* is distributed throughout the Old and the New World, whereas the subgenus *Viannia* is only found in the New World.¹⁶ In the Western Hemisphere, multiple species regularly infect people: *Leishmania (Leishmania) amazonensis*, *Leishmania (Viannia) braziliensis*, *L. (V.) peruviana*, *L. (V.) colombiensis*, *L. (L.) donovani*, *L. (L.) garnhami*, *L. (V.) guyanensis*, *L. (L.) infantum* (Old World)/*L. (L.) chagasi* (New World), *L. (V.) lainsoni*, *L. (V.) lindenbergi*, *L. (L.) mexicana*, *L. (V.) naiffi*, *L. (V.) panamensis*, *L. (L.) pifanoi*, *L. (V.) shawi*, and *L. (L.) venezualensis*. In the Eastern Hemisphere, there are significantly fewer species that infect humans: *L. (L.) donovani*, *L. (L.) infantum*, *L. (L.) aethiopica*, *L. (L.) major*, and *L. (L.) tropica*.

The clinical conditions caused by *Leishmania* spp. vary greatly, depending upon the species of *Leishmania* and the host's immune status. The disease can present as cutaneous lesions that resolve over time or as a systemic disease of the reticuloendothelial system, which often results in the host's death if left untreated. Fortunately, there are fewer clinical entities than the number of species of pathogens that cause them: VL, CL, MCL, DCL, and, less commonly, LR.

This introductory chapter will summarize the biology and molecular biology of the entire group, as they all behave similarly within their intracellular environment and sand fly vectors. Exceptions will be presented whenever they relate to a disease process applicable only to that species. The *Leishmania* Genome Project is based on the genome of *Leishmania major*, and in 2005, the full genomic sequence was completed.¹⁷

There are no commercially available vaccines yet, but infection with many *Leishmania* species usually results in immunity to reinfection with the same species.^{18, 19} Perhaps data from the genome project should help hasten the development of a vaccine against the most dangerous forms of leishmaniasis. Developing a vaccine for both animals and humans is an active area of research.²⁰

Life Cycle

The sand fly

Infection of the sandfly begins when the insect obtains blood from an infected mammal. Only female sand flies, mostly active during dawn and dusk and nighttime hours (crepuscular/nocturnal pattern), feed on blood.^{13, 21} A blood meal is a requirement for egg production. The fly injects saliva containing numerous well-characterized bioactive components, many of which are peptides or proteins.^{22, 23} One such protein,

maxadilan (a potent vasodilator), is a seven kDa peptide believed to be essential to taking a blood meal by the fly.²⁴ Maxadilan's primary mode of action is to reduce intracellular calcium in the host at the bite site through a cAMP-dependent mechanism, causing arterial dilation.^{25, 26} Blood can then quickly be drawn up by the insect. The receptor for maxadilan is the pituitary adenylate cyclase-activating polypeptide, a membrane-bound protein found on many cell types in the body, including smooth muscle cells and macrophages.²⁵ During feeding, infected sandflies become maximally filled with blood. They cannot regurgitate the excess due to inhibiting the emptying reflex by a parasite-specific peptide that interacts with myosin to prevent stomach muscle contraction.²⁷ This enhances the chances for the sand fly to become infected and remain so throughout the period that the parasite needs (1–2 weeks) to develop into the infectious stage for a mammalian host.

The parasite undergoes a complex series of developmental changes inside the gut tract of the sand fly, taking about one week to progress to the flagellated metacyclic stage.² *Leishmania* first attaches to the wall of the gut tract by non-specific hydrophobic interactions between the surface of the parasite's flagella and the insect's stomach cell membrane.²⁸ Attachment to other regions of the insect's intestinal tract later on during the differentiation to the metacyclic promastigote stage is mediated, in part, by specific insect galectins (e.g., PpGalec), and the parasite cell surface multipurpose molecule, lipophosphoglycan.^{29, 30} The release of infectious stage organisms, a necessary final step in their development, is mediated by arabinocyl capping of lipophosphoglycan social residues upon differentiation to the metacyclic stage.³¹ The leptomastigote stage locates to the anterior region



Figure 2.2. Promastigotes of *Leishmania* spp., as seen in culture.

of the gut. It secretes a gel-like substance that blocks the digestive tract of the sand fly, causing the infected insect to regurgitate its complement of infectious metacyclic promastigotes into the host's subcutaneous tissues during feeding.²

The mammalian host

The flagellated metacyclic promastigote stage (Fig. 2.2) resides in the anterior midgut and thorax and is injected into the host along with the fly's salivary secretions. Some of those same salivary proteins aid in *Leishmania*'s ability to colonize the mammalian host.^{32, 33} Following injection of the metacyclic promastigote stage, there is a rapid infiltration of neutrophils into the skin.³⁴ Several types of tissue-resident phagocytic cells quickly take up the promastigotes.³⁵ Maxadilan, produced by the parasite, induces adverse effects on host immune cell function, including inhibition of the release of TNF- α , upregulation of interleukin-6 synthesis in macrophages, increase in interleukin-10 production, and stimulation of prostaglandin E2 production.³⁶ This leads to a down-regulation of Th1-type cytokines and a shift to a Th2 response.³⁷

The promastigotes deposited in the extracellular matrix at the bite site adhere there, aided by lipophosphoglycan and a surface membrane laminin receptor protein on their surface.³⁸ The promastigotes induce the production of antibodies and become opsonized. As a result, the complement C3 protein attaches to the parasite cell surface.³⁹ The promastigotes can then attach to red cells or platelets and become engulfed by dendritic cells or macrophages (Fig. 2.3). Many would-be pathogens cannot survive this step and are digested by phagolysosomal vacuoles. In contrast, *Leishmania* avoid digestion and are free to differentiate into amastigotes to begin the intracellular phase of their life cycle due to their ability to inhibit phagolysosome maturation. Phagolysosome biogenesis is inhibited largely by the promastigote surface lipophosphoglycan.^{41 42} Infected phagocytes display abnormal phagolysosome maturation due to lipophosphoglycan's interference with F-actin, an essential component of the process of fusion of lysosomes with the phagocytic vacuole.⁴³ This lack of fusion enables the parasite to evade digestion.

At this point in the life cycle, differences between species of *Leishmania* become apparent. Those that cause only cutaneous lesions remain at the site throughout the infection. In contrast, those that cause visceral or mucocutaneous lesions find their

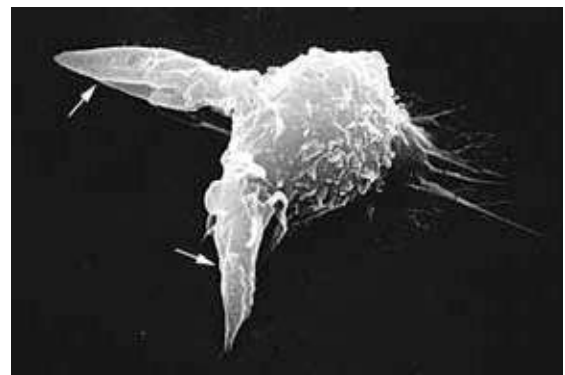


Figure 2.3. Scanning EM of macrophage ingesting two promastigotes (arrows). Courtesy K-P Chang.

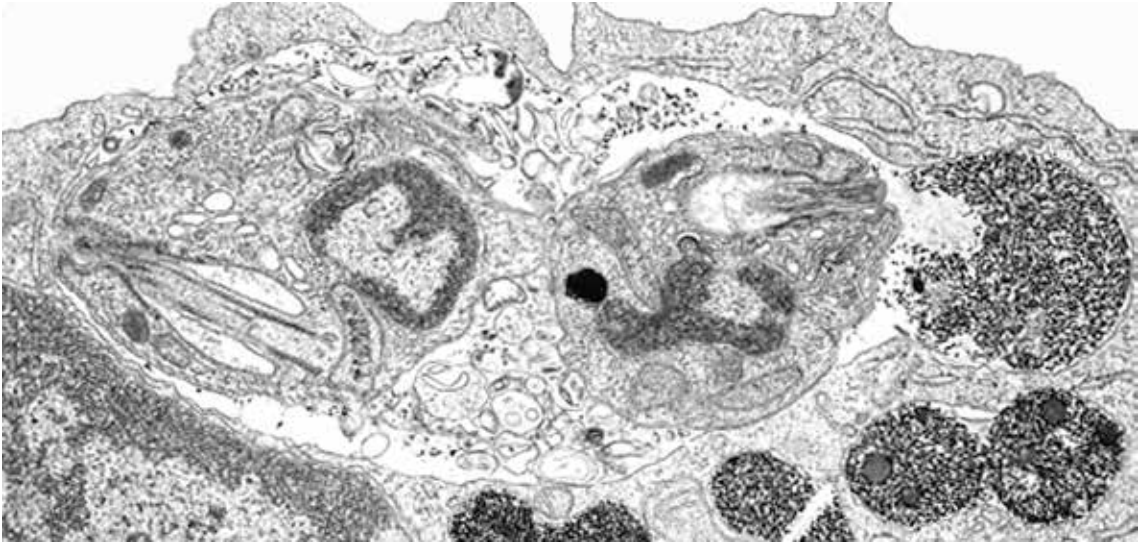


Figure 2.4. Electron micrograph of two amastigotes of *Leishmania* spp. Courtesy K-P Chang.

way to the appropriate location in the body. The host and parasite factors resulting in these different infection strategies are still under investigation. For example, dendritic cells increase in number in the draining lymph nodes of experimentally infected mice infected with *L. (L.) tropica*. Infected dendritic cells do not appear to migrate to the lymph nodes. How the parasites reach the draining lymphoid tissue remains to be demonstrated.

Amastigotes divide inside their host cells (Fig. 2.4) and can remain at the injection site, resulting in the clinical condition known as CL. Alternatively, the phagocytes can carry them to mucocutaneous junctions or reticuloendothelial tissues, resulting in MCL or VL. Cutaneous lesions form in most instances, allowing sand flies access to infected host cells at the raised margin. While feeding, the vector can also take up circulating macrophages that harbor amastigotes.

Cellular and Molecular Pathogenesis

Virulence factors and pathogenesis

The cellular and molecular biology of

Leishmania spp. and the complexity of its interaction with the host's innate and adaptive immune system has been the subject of many extensive reviews, which suggest that a better understanding of this interaction may lead to improved therapeutics.^{9, 44, 45} Most of what is known regarding the biology of *Leishmania* is derived from murine models and *in vitro* cell culture using various species of *Leishmania*.⁴⁶ Notably, there is a lack of nasopharyngeal pathology in mice, favoring hamster models for investigating MCL.⁴⁷

The following summary of pathogenic mechanisms is derived from both experimental approaches. The turning on of heat shock genes and cassettes of other developmentally regulated genes occurs as the parasite transitions from an environment dependent upon ambient temperature (sand fly) to the homeothermic essential niche inside the mammalian host cell.^{48, 49} The amastigote down-regulates interleukin-12, which delays the onset of cell-mediated protective immune responses.^{50, 51} Amastigotes have been shown to interfere with antigen presentation by macrophages, employing cysteine protease B.⁵² The amastigote stage also possesses potent cysteine protease

inhibitors, presumably used to modify host cysteine protease activity during intracellular infection.⁵³

Amastigotes' replication appears to depend on host cyclophilins since division is inhibited by cyclosporine A.^{54, 55} The membrane of the promastigote contains a zinc protease, leishmanolysin, a 63 kDa glycoprotein whose crystalline structure has been determined.^{56, 57} Current evidence favors the role of leishmanolysin in the migration of parasites through the extracellular matrix after their release from infected cells by digestion of collagen type IV.⁵⁸ Induction of the chemokine MIP-1 β by neutrophils harboring amastigotes attracts macrophages to the site of infection. Macrophages then engulf infected neutrophils, thus acquiring the infection.⁵⁹

An exciting advance in our understanding of the severity of certain forms of mucocutaneous disease was the discovery of a double-stranded RNA virus that belongs to the Totiviridae family and infects the *L. Viania* subgenus.⁶⁰ It appears that this *Leishmania* RNA Virus (LRV) is recognized by host toll-like receptors and induces an inflammatory response that leads to a hyper-inflammatory immune response with resultant destructive lesions.^{60, 61}

Protective immune mechanism(s)

The mechanism(s) of protective immunity vary with clinical types of *Leishmania*.⁶² The cutaneous forms typically induce well-defined Th1 responses, which are T-cell mediated and play a critical role in controlling and finally eliminating the organism.⁶³ Immunity to reinfection with CL-causing organisms is the rule, species-specific, and depends upon inducing and maintaining high levels of cytotoxic and memory

T cells.⁶⁴ In addition, Langerhans cells are thought to play a significant role in antigen presentation and the induction of interleukin-12 and interleukin-27.⁶⁵⁻⁶⁷ The primary effector mechanism involves cytotoxic T helper cell-dependent macrophage activation and subsequent killing of amastigotes by NO.⁶⁸ Chemokines are also crucial for immunity, including macrophage inflammatory protein 1 β -3 β and interferon- γ .^{69, 70} Antibodies appear to play no role in immunity to CL and probably aid the parasite in gaining entrance into the macrophage.⁷¹ Protective immune mechanisms induced by infection with VL (*L. (L.) donovani* and *L. (L.) infantum*) include interleukin-12 and interferon- γ . Immunity is suppressed by interleukin-10 and transforming growth factor- β .⁶³

Further complicating the clinical spectrum of diseases caused by *Leishmania* is how long the *Leishmania* spp. have been around. Within the last 165 million years, they have diverged evolutionarily due to geographical isolation caused by continental drift. Organisms in the New World have evolved somewhat differently from their ancestor species that continue to infect mammals in the Old World. The same is true for the hosts. Many exceptions exist to the above summaries when considering the type of disease and the immune responses to them. There are excellent reviews on *Leishmania*,^{13, 72} and the WHO NTD roadmap highlights critical factors to control and eliminate disease, including vector control and early detection, which enable prompt treatment in children and young adults. In addition, point-of-care diagnostics are needed, particularly in East Africa, with ready access to medicines most likely to ensure a cure with the least number of adverse drug reactions.⁷³

References

1. Mauricio, I. L., et al., Genomic diversity in the *Leishmania donovani* complex. *Parasitology* **1999**, *119* (Pt 3), 237-46.
2. Bates, P. A.; Rogers, M. E., New insights into the developmental biology and transmission mechanisms of *Leishmania*. *Current molecular medicine* **2004**, *4* (6), 601-9.
3. Bates, P. A., Transmission of *Leishmania* metacyclic promastigotes by Phlebotomine sand flies. *Int J Parasitol* **2007**, *37* (10), 1097-106.
4. de Souza, A., et al., Psychodopygus complexus, a new vector of *Leishmania braziliensis* to humans in Para State, Brazil. *Trans R Soc Trop Med Hyg* **1996**, *90* (2), 112-3.
5. Brilhante, A. F., et al., A New Species of Sand Fly, *Psathyromyia elizabethdorvalae* sp. n. (Diptera: Psychodidae: Phlebotominae), From Brazil. *J Med Entomol* **2017**, *54* (1), 76-81.
6. Ashford, R. W., The leishmaniasis as model zoonoses. *Annals of tropical medicine and parasitology* **1997**, *91* (7), 693-701.
7. Quinnell, R. J.; Courtenay, O., Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitology* **2009**, *136* (14), 1915-34.
8. Moyersoen, J., et al., Biogenesis of peroxisomes and glycosomes: trypanosomatid glycosome assembly is a promising new drug target. *FEMS microbiology reviews* **2004**, *28* (5), 603-43.
9. Kaye, P.; Scott, P., Leishmaniasis: complexity at the host-pathogen interface. *Nat Rev Microbiol* **2011**, *9* (8), 604-15.
10. Marovich, M. A., et al., Leishmaniasis recidivans recurrence after 43 years: a clinical and immunologic report after successful treatment. *Clin Infect Dis* **2001**, *33* (7), 1076-9.
11. Guimaraes, L. H., et al., Atypical Manifestations of Cutaneous Leishmaniasis in a Region Endemic for *Leishmania braziliensis*: Clinical, Immunological and Parasitological Aspects. *PLoS Negl Trop Dis* **2016**, *10* (12), e0005100.
12. Reithinger, R., et al., Cutaneous leishmaniasis. *Lancet Infect Dis* **2007**, *7* (9), 581-96.
13. Mann, S., et al., A Review of Leishmaniasis: Current Knowledge and Future Directions. *Curr Trop Med Rep* **2021**, *8* (2), 121-132.
14. Curtin, J. M.; Aronson, N. E., Leishmaniasis in the United States: Emerging Issues in a Region of Low Endemicity. *Microorganisms* **2021**, *9* (3).
15. Hotez, P. J., The rise of neglected tropical diseases in the “new Texas”. *PLoS Negl Trop Dis* **2018**, *12* (1), e0005581.
16. Dutari, L. C.; Loaiza, J. R., American Cutaneous Leishmaniasis in Panama: a historical review of entomological studies on anthrophilic *Lutzomyia* sand fly species. *Parasit Vectors* **2014**, *7*, 218.
17. Ivens, A. C., et al., The genome of the kinetoplastid parasite, *Leishmania major*. *Science* **2005**, *309* (5733), 436-42.
18. Dinc, R., *Leishmania* Vaccines: the Current Situation with Its Promising Aspect for the Future. *Korean J Parasitol* **2022**, *60* (6), 379-391.
19. Handman, E., Leishmaniasis: current status of vaccine development. *Clinical microbiology reviews* **2001**, *14* (2), 229-43.
20. Kumar, R.; Engwerda, C., Vaccines to prevent leishmaniasis. *Clin Transl Immunology* **2014**, *3* (3), e13.
21. Lestonova, T., et al., Insights into the sand fly saliva: Blood-feeding and immune interactions between sand flies, hosts, and *Leishmania*. *PLoS Negl Trop Dis* **2017**, *11* (7), e0005600.
22. Valenzuela, J. G., et al., Identification of the most abundant secreted proteins from the salivary glands of the sand fly *Lutzomyia longipalpis*, vector of *Leishmania chagasi*. *The Journal of experimental biology* **2004**, *207* (Pt 21), 3717-29.
23. Dominguez, M., et al., Early mechanisms of *Leishmania* infection in human blood. *Microbes Infect* **2003**, *5*, 507-13.
24. Jackson, T. S., et al., Vasodilatory properties of recombinant maxadilan. *The American journal of physiology* **1996**, *271* (3 Pt 2), H924-30.
25. Moro, O.; Lerner, E. A., Maxadilan, the vasodilator from sand flies, is a specific pituitary adenylate cyclase activating peptide type I receptor agonist. *The Journal of biological chemistry* **1997**, *272*

- (2), 966-70.
26. Uchida, D., et al., Maxadilan is a specific agonist and its deleted peptide (M65) is a specific antagonist for PACAP type 1 receptor. *Annals of the New York Academy of Sciences* **1998**, 865, 253-8.
27. Vaidyanathan, R., Isolation of a myoinhibitory peptide from *Leishmania major* (Kinetoplastida: Trypanosomatidae) and its function in the vector sand fly *Phlebotomus papatasi* (Diptera: Psychodidae). *Journal of medical entomology* **2005**, 42 (2), 142-52.
28. Wakid, M. H.; Bates, P. A., Flagellar attachment of *Leishmania* promastigotes to plastic film *in vitro*. *Experimental parasitology* **2004**, 106 (3-4), 173-8.
29. Kamhawi, S., et al., A role for insect galectins in parasite survival. *Cell* **2004**, 119 (3), 329-41.
30. Beverley, S. M.; Dobson, D. E., Flypaper for parasites. *Cell* **2004**, 119 (3), 311-2.
31. Dobson, D. E., et al., Identification of genes encoding arabinosyltransferases (SCA) mediating developmental modifications of lipophosphoglycan required for sand fly transmission of *Leishmania major*. *The Journal of biological chemistry* **2003**, 278 (31), 28840-8.
32. Ribeiro, J. M., Blood-feeding arthropods: live syringes or invertebrate pharmacologists? *Infectious agents and disease* **1995**, 4 (3), 143-52.
33. Nuttall, P. A., et al., Vector-host interactions in disease transmission. *Journal of molecular microbiology and biotechnology* **2000**, 2 (4), 381-6.
34. Peters, N. C., et al., *In vivo* imaging reveals an essential role for neutrophils in leishmaniasis transmitted by sand flies. *Science* **2008**, 321 (5891), 970-4.
35. Ng, L. G., et al., Migratory dermal dendritic cells act as rapid sensors of protozoan parasites. *PLoS Pathog* **2008**, 4 (11), e1000222.
36. Soares, M. B., et al., The vasoactive peptide maxadilan from sand fly saliva inhibits TNF-alpha and induces IL-6 by mouse macrophages through interaction with the pituitary adenylate cyclase-activating polypeptide (PACAP) receptor. *Journal of immunology (Baltimore, Md. : 1950)* **1998**, 160 (4), 1811-6.
37. Brodie, T. M., et al., Immunomodulatory effects of the *Lutzomyia longipalpis* salivary gland protein maxadilan on mouse macrophages. *Infect Immun* **2007**, 75 (5), 2359-65.
38. Ghosh, A., et al., Isolation of a laminin-binding protein from the protozoan parasite *Leishmania donovani* that may mediate cell adhesion. *The Biochemical journal* **1999**, 337 (Pt 3), 551-8.
39. Antoine, J.-C., et al., *Leishmania* spp.: on the interactions they establish with antigen-presenting cells of their mammalian hosts. *Advances in parasitology* **2004**, 58, 1-68.
40. Steigerwald, M.; Moll, H., *Leishmania major* modulates chemokine and chemokine receptor expression by dendritic cells and affects their migratory capacity. *Infection and immunity* **2005**, 73 (4), 2564-7.
41. Moradin, N.; Descoteaux, A., *Leishmania* promastigotes: building a safe niche within macrophages. *Front Cell Infect Microbiol* **2012**, 2, 121.
42. Turco, S. J.; Descoteaux, A., The lipophosphoglycan of *Leishmania* parasites. *Annual review of microbiology* **1992**, 46, 65-94.
43. Lodge, R.; Descoteaux, A., Modulation of phagolysosome biogenesis by the lipophosphoglycan of *Leishmania*. *Clinical immunology (Orlando, Fla.)* **2005**, 114 (3), 256-65.
44. K.J., G., The biology of kinetoplastid parasites: insights and challenges from genomics and post-genomics. *Int* **2001**, 31, 443-52.
45. Olivier, M., et al., Subversion mechanisms by which *Leishmania* parasites can escape the host immune response: a signaling point of view. *Clinical microbiology reviews* **2005**, 18 (2), 293-305.
46. Debrabant, A., et al., Generation of *Leishmania donovani* axenic amastigotes: their growth and biological characteristics. *International journal for parasitology* **2004**, 34 (2), 205-17.
47. Olivier, M., Host-pathogen interaction: Culprit within a culprit. *Nature* **2011**, 471 (7337), 173-4.
48. Bente, M., et al., Developmentally induced changes of the proteome in the protozoan parasite *Leishmania donovani*. *Proteomics* **2003**, 3 (9), 1811-29.
49. Duncan, R. C., et al., The application of gene expression microarray technology to kinetoplastid research. *Current molecular medicine* **2004**, 4 (6), 611-21.
50. Sutterwala, F. S.; Mosser, D. M., The taming of IL-12: suppressing the production of proinflammatory cytokines. *Journal of leukocyte biology* **1999**, 65 (5), 543-51.
51. McDowell, M. A.; Sacks, D. L., Inhibition of host cell signal transduction by *Leishmania*:

- observations relevant to the selective impairment of IL-12 responses. *Current opinion in microbiology* **1999**, 2 (4), 438-43.
52. Buxbaum, L. U., et al., Cysteine protease B of *Leishmania mexicana* inhibits host Th1 responses and protective immunity. *Journal of immunology (Baltimore, Md. : 1950)* **2003**, 171 (7), 3711-7.
 53. Besteiro, S., et al., A potential role for ICP, a Leishmanial inhibitor of cysteine peptidases, in the interaction between host and parasite. *Molecular microbiology* **2004**, 54 (5), 1224-36.
 54. Hoerauf, A., et al., Host-cell cyclophilin is important for the intracellular replication of *Leishmania major*. *Molecular microbiology* **1997**, 24 (2), 421-9.
 55. Meissner, U., et al., Cyclosporin A-mediated killing of *Leishmania major* by macrophages is independent of reactive nitrogen and endogenous TNF-alpha and is not inhibited by IL-10 and 13. *Parasitol Res* **2002**, 89, 221-7.
 56. Chaudhuri, G., et al., Surface acid proteinase (gp63) of *Leishmania mexicana*. A metalloenzyme capable of protecting liposome-encapsulated proteins from phagolysosomal degradation by macrophages. *The Journal of biological chemistry* **1989**, 264 (13), 7483-9.
 57. Schlagenhauf, E., et al., The crystal structure of the *Leishmania* major surface proteinase leishmanolysin (gp63). *Structure (London, England : 1993)* **1998**, 6 (8), 1035-46.
 58. McGwire, B. S., et al., Migration through the extracellular matrix by the parasitic protozoan *Leishmania* is enhanced by surface metalloprotease gp63. *Infection and immunity* **2003**, 71 (2), 1008-10.
 59. van Zandbergen, G., et al., Cutting edge: neutrophil granulocyte serves as a vector for *Leishmania* entry into macrophages. *Journal of immunology (Baltimore, Md. : 1950)* **2004**, 173 (11), 6521-5.
 60. Ives, A., et al., Leishmania RNA virus controls the severity of mucocutaneous leishmaniasis. *Science* **2011**, 331 (6018), 775-8.
 61. Parra-Munoz, M., et al., Detection of Leishmania RNA Virus in Clinical Samples from Cutaneous Leishmaniasis Patients Varies according to the Type of Sample. *Am J Trop Med Hyg* **2021**, 104 (1), 233-239.
 62. Wilson, M. E., et al., Immunopathogenesis of infection with the visceralizing *Leishmania* species. *Microbial pathogenesis* **2005**, 38 (4), 147-60.
 63. Scott, P., et al., The development of effector and memory T cells in cutaneous leishmaniasis: the implications for vaccine development. *Immunological reviews* **2004**, 201, 318-38.
 64. Gabaglia, C. R., et al., Life-long systemic protection in mice vaccinated with *L. major* and adenovirus IL-12 vector requires active infection, macrophages and intact lymph nodes. *Vaccine* **2004**, 23 (2), 247-57.
 65. Oliveira, M. A. P., et al., Macrophages at intermediate stage of maturation produce high levels of IL-12 p40 upon stimulation with *Leishmania*. *Microbes and infection / Institut Pasteur* **2005**, 7 (2), 213-23.
 66. Simin, M.; Shahriar, D., A quantitative study of epidermal Langerhans cells in cutaneous Leishmaniasis caused by *Leishmania tropica*. *International Journal of Dermatology* **2004**, 43, 819-823.
 67. Hunter, C. A., et al., The role of IL-27 in the development of T-cell responses during parasitic infections. *Immunological reviews* **2004**, 202, 106-14.
 68. Awasthi, A., et al., Immune response to *Leishmania* infection. *The Indian journal of medical research* **2004**, 119 (6), 238-58.
 69. Roychoudhury, K.; Roy, S., Role of chemokines in *Leishmania* infection. *Current molecular medicine* **2004**, 4 (6), 691-6.
 70. Mitra, R., et al., Migration of antigen presenting cells from periphery to the peritoneum during an inflammatory response: role of chemokines and cytokines. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **2004**, 18 (14), 1764-6.
 71. Miles, S. A., et al., A role for IgG immune complexes during infection with the intracellular pathogen *Leishmania*. *The Journal of experimental medicine* **2005**, 201 (5), 747-54.
 72. McMahon-Pratt, D.; Alexander, J., Does the *Leishmania major* paradigm of pathogenesis and protection hold for New World cutaneous leishmaniases or the visceral disease? *Immunological reviews* **2004**, 201, 206-24.
 73. Organization, W. H., Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva. Licence: CC BY-NC-SA 3.0 IGO **2020**.

3. Cutaneous Leishmaniasis

Pronunciation: \lēsh-'ma-nē-ə\

Leishmania (L) major

(Yakimov and Schockov 1915)

Leishmania (L) tropica

(Wright 1903)

Leishmania (L) mexicana

(Biagi 1953)

Introduction

Cutaneous leishmaniasis (CL) is a complicated disease due to the large number of species and clinical variants. Roughly speaking, we consider CL divided into Old World CL, meaning it's found predominantly in the Middle East and North Africa (MENA) region, East Africa, South Asia and Central Asia, and New World CL in the Americas. Each of these species varies with respect to its geographic distribution, clinical manifestations, sand fly intermediate host, and whether it is zoonotic. Zoonotic species, such as dogs or rodents, require a significant animal reservoir.¹ Anthroponotic species cycle only or predominantly between humans and sand flies (e.g., *Leishmania (L) tropica*, (LEESH-ma-NEE-ah)).

Four species cause Old World CL: *Leishmania (L.) aethiopica*, *L. (L.) major*, *L. (L.) tropica*, and *L. (L.) infantum*. Their vectors include sand flies of the following species: *Phlebotomous papatasi*, *P. sergenti*, *P. longipes*, *P. argentipes*, and *P. ariasi*. At least 15 species of *Leishmania* in the New World cause similar types of disease: *Leishmania (Leishmania) amazonensis*, *L. (V.) braziliensis*, *L. (V.) colombiensis*, *L. (L.) garnhami*, *L. (V.) guyanensis*, *L. (L.) infantum*, *L. (V.) lainsoni*, *L. (V.) lindenbergi*, *L. (L.) mexicana*, *L. (V.) naiffi*, *L. (V.) panamensis*, *L. (V.) peruviana*, *L. (L.) pifanoi*, *L. (V.) shawi*, *L. (L.) venezuelensis*. The principal vector species

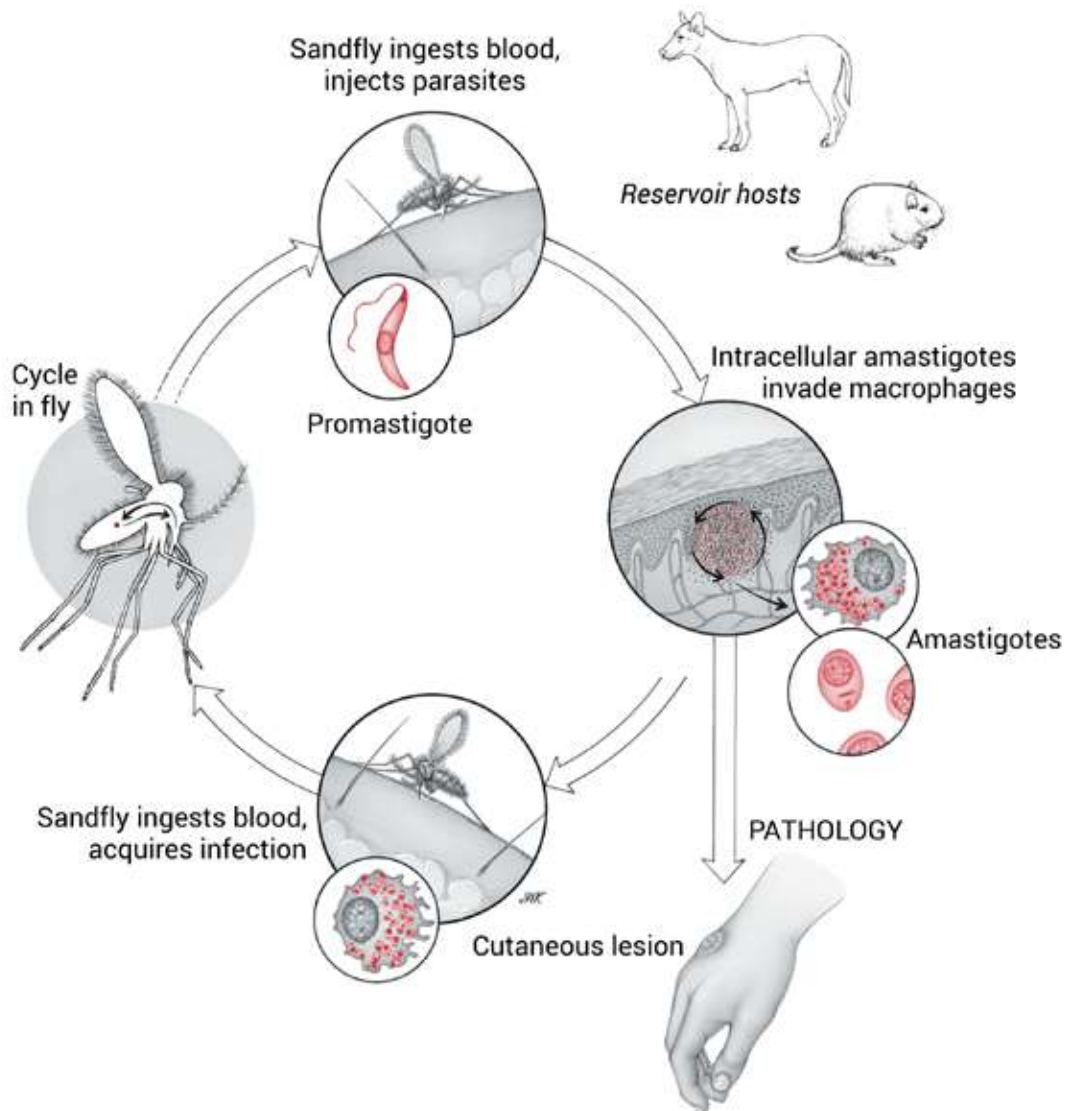
are *Lutzomyia olmeca olmeca*, *Lu. flaviscutellata*, and *Lu. trapidoi*. In some locales and some populations, *L. (L.) tropica* visceralizes, as was seen in soldiers returning from Operation Desert Storm (1990–1991), suggesting that another strain of this species exists with quite different characteristics from the one that only causes cutaneous lesions or, more likely, that host factors such as genetic backgrounds or immune history may influence the type of disease that manifests.^{2,3}

Although accurate statistics regarding incidence rates and prevalence are not available, there are estimated to be 0.7–1.2 million cases of CL each year.⁴ Rodents are the primary reservoir for human infection caused by *L. (L.) major*. Domestic dogs serve as reservoirs in many parts of the world for other species of *Leishmania*.⁵ Old World CL cases are often linked to the breakdown in public health infrastructure resulting from conflict.⁶ Estimates are difficult to come by due to the absence of public health surveillance in these regions, but it is believed that the numbers could be in the hundreds of thousands.



Figure 3.1. A lesion on face due to *Leishmania major*.

Leishmania tropica



New World CL is also linked to conflict and human migrations, including cases connected with guerilla movements, narcotrafficking in South America, and a Cuban diaspora through the Darien jungle of Panama.^{7,8}

Historical Information

In 1756, Alexander Russell described the clinical aspects of CL.⁹ A few years later, in 1764, Cosme Bueno suspected that “uta” was a form of CL and was later shown to be caused by infection with *Leishmania*.¹⁰ It was not until a hundred years later, in 1885, while working in India, that David Cunningham accurately described the *Leishmania* organism he saw in a fixed histological section of a skin lesion.¹¹ In 1898, Peter Borovsky, a Russian military surgeon, visualized this organism in lesions and correctly identified it as a protozoan pathogen causing CL. In 1921, Edouard and Etienne Sergent demonstrated that sandflies were the vectors responsible for transmitting *Leishmania* to humans. One species bears their name, *P. sergenti*.

“Oriental sore” is common among people living in endemic areas of the Middle East, India, and Africa. A rudimentary kind of immunization referred to as “leishmanization” was practiced in the Middle East, where it was known that infection results in permanent immunity to reinfection.¹² Uninfected individuals were deliberately inoculated in areas other than the face with scrapings containing organisms from the margins of active lesions. This controlled the region of the body on which the scar developed.¹³ Unfortunately, many individuals would develop chronic lesions that did not heal due to these inoculations, and much effort is being put into alternative approaches.¹⁴

Life Cycle

Infection begins with the bite of an infected sandfly. The promastigotes are introduced into the subcutaneous tissue, attach to the extracellular matrix, and then taken up by dendritic cells and macrophages. The promastigotes transform into the amastigote stage and begin to replicate. The infection progresses only at the site of the bite. Eventually, a large, painless craterform ulcer develops due to extensive cell death.¹⁵ Sand flies acquire the infection by feeding on blood taken up at the margin of the ulcer.¹⁶

Clinical Disease

CL is first recognized as a small red papule at the bite wound site approximately 2–8 weeks after injection of metacyclic promastigotes. The lesion progresses from a painless nodule, measuring about 1 cm in diameter, into a larger one (Fig. 3.1). The area around the bite wound eventually ulcerates due to the intense destruction of cells. It becomes depressed or, in some cases, raised (Fig. 3.2). Organisms are found only in the living tissue at the raised margin, regardless of the age of the lesion (Fig. 3.3). Occasionally, more than one lesion develops (Fig. 3.4).

After the ulcer heals, which may take weeks



Figure 3.2. Exophytic lesion due to *Leishmania* spp.

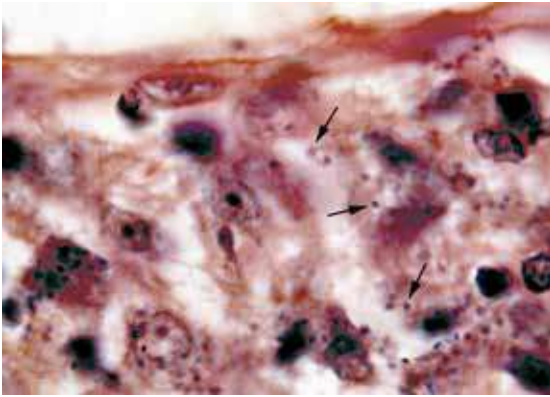


Figure 3.3. Histologic section of skin showing amastigotes (arrows) of *Leishmania* spp. in dendritic cells and macrophages.

to months, immunity to reinfection is usually permanent. Although generally species-specific, it may offer some protection against other *Leishmania* species.¹³ In experimental infections in rodents, exposure to *L. (V.) braziliensis* confers protection against challenge with *L. (L.) major*, suggesting that shared, cross-reacting antigens might be good candidates for a CL vaccine.^{17, 18}

Regardless of which species causes the lesion, it may vary in size and shape, sometimes confounding even the most experienced clinician. CL should be considered in travelers or patients from endemic areas when encountering a lesion that fails to heal.¹⁹ This is particularly true for CL in the Western Hemisphere.²⁰ Prolonged infection is the rule in patients with an altered immunity pattern.²¹ Chiclero's ulcer on the ear's pinna (Fig. 3.5) is an excellent example of this exception.²²

L. (L.) aethiopica infection is restricted to Ethiopia and Kenya. It causes a broad spectrum of diseases, including diffuse cutaneous leishmaniasis (DCL), characterized by involvement of the entire skin surface.²³⁻²⁵ Other species of *Leishmania* (e.g., *L. (V.) braziliensis*, *L. (L.) amazonensis*) have also been diagnosed in patients suffering from DCL.²⁶ DCL begins as a single nodule on an exposed part of the skin, then starts to grow and spread, eventually involving vast areas of the skin. Patients can resemble those suf-

fering from various forms of leprosy.²⁷

Similarly, *L. (L.) mexicana*, *L. (L.) amazonensis*, and *L. (L.) venezuelensis* occasionally cause anergic disseminated cutaneous leishmaniasis (ADCL), which is similar to DCL.²⁸ Nodules and large patches of involved skin, in which abundant amastigote-infected cells can be found, are typical and may remain for many years. Chemotherapy is of little consequence to the outcome, which includes extensive scarring of all involved areas. However, patients may benefit from newer treatment approaches.²⁹⁻³¹ *Leishmania (L.) infantum* can cause cutaneous lesions but is more commonly associated with visceral disease in children throughout the Mediterranean basin.¹⁵



Figure 3.4. Multiple cutaneous lesions due to *Leishmania panamanensis*.



Figure 3.5. Chiclero's ulcer due to *Leishmania*.

Patients with HIV/AIDS may present with cutaneous lesions only, or the infection can visceralize.²⁰ While cutaneous lesions are not frequently described in the HIV-negative population with visceral leishmaniasis (VL), multiple patients co-infected with *Leishmania* and HIV have been reported with cutaneous lesions containing *Leishmania* organisms.³²

Diagnosis (see Clinical Appendix)

The typical lesion in CL is a nodule that enlarges into a painless ulcer with an indurated border. Although most cutaneous lesions caused by *Leishmania* look similar (i.e., crateriform with a raised edge), many other dermatological conditions might be mistaken for CL. Diagnosis of all forms of CL due to *Leishmania* is best achieved by isolating the organism or through NAAT. Samples should be taken from the margin of an active ulcer that is not superinfected.³³ The edge can be scraped gently with a scalpel to obtain material, an aspiration can be performed, or a full-thickness biopsy can be obtained from the ulcer edge, and a touch

prep can be performed before sending specimens off for histology, culture, microscopy, or NAAT.³³ Non-invasive techniques such as filter paper lesion impressions (FPLIs) and cytology brushes have also shown comparable sensitivities and specificities to invasively obtained specimens.³⁴⁻³⁶

NAAT techniques have high sensitivities and also offer the advantage of species identification.³⁷⁻⁴¹ Species identification is critical in determining whether the infecting species is *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis*, and *L. (V.) amazonensis*, which have the potential to progress to mucocutaneous disease.⁴² Histopathological examination of biopsy tissue has less sensitivity than NAAT techniques. It requires recognition of the characteristic amastigote tissue form of the parasite with the nucleus and kinetoplast (the *dot and dash*).⁴³ The culture of needle aspiration samples, scrapings, and biopsy specimens is a viable alternative to NAAT. Still, it takes much longer (several days) due to the slow growth of the promastigotes at 20 °C. Laboratories with the most experience in parasite culture generally have the highest success rates.⁴⁴⁻⁴⁶

Serology is not routinely used to diagnose CL, but antibody testing has been used in some instances to evaluate patients from non-endemic areas.⁴⁷ The leishmaniasis skin test is used in certain parts of the world. It involves injecting killed promastigotes intradermally and is read at 48–72 hours, much like the purified protein derivative (PPD) skin test for tuberculosis infection.⁴⁸ An interferon-release assay was developed for leishmaniasis, and although not in routine clinical use, it has been used for epidemiologic outbreak investigations.⁵

Treatment (see Clinical Appendix)

The severity of the lesion, its location, and the species involved dictate the treatment of CL. Uncomplicated infections not involving species associated with mucosal disease may be treated with local therapy, as many resolve clinically without treatment. It is important to note that the goal of therapy is clinical cure and not complete clearance of parasites, as some parasites will persist even with successful treatment.^{51,52} In most cases, spontaneous healing will occur with proper hygiene and wound care.^{53,54}

Cryotherapy using liquid nitrogen has been successfully employed.⁵⁵⁻⁵⁷ Treatment with thermotherapy delivered by radiofrequency, superficial heat at 50 °C, or application of heated prongs can result in a clinical cure. Still, it must be used cautiously to avoid nerve damage and burns.^{58,59} Topical paromomycin and intra-lesional sodium stibogluconate (pentavalent antimony) are alternative but less-studied approaches. Intra-lesional injections of pentavalent antimony are not approved in the United States and are associated with significant pain.⁶⁰⁻⁶²

Several systemic therapies are in use for the treatment of CL, including pentavalent antimonials (sodium stibogluconate or meglumine antimoniate), amphotericin, miltefosine, pentamidine, and azole drugs.⁶³ Systemic therapy is recommended if the infecting species can cause mucosal disease based on species identification, as can occur with the acquisition of the infection in what is referred to as the mucosal belt (Brazil, Bolivia, and portions of Peru).⁶⁴ Identification of subspecies guides therapy as *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis*, and *L. (V.) amazonensis* can cause mucocutaneous disease.⁶⁵ It is estimated that without treatment, the different rates of mucocutaneous disease for the other subspecies are 10-30% for *L. (V.) braziliensis*, 10% for *L. (V.) guyanensis*, less than 10% for *L. (V.) panamensis*,

and rarely for *L. (V.) amazonensis*. Sodium stibogluconate, an antimony-containing drug with many serious side effects, including rash, headache, arthralgias and myalgias, pancreatitis, transaminitis, and hematologic suppression, is the drug of choice in many parts of the world, in part due to its relatively low cost. Still, increasing rates of resistance have already limited its use.⁶⁶ Amphotericin B and its liposomal form are alternative and highly effective options for treatment.⁶⁷ In the developed world, liposomal amphotericin B is a frequent choice of therapy, but its cost is prohibitive in many areas where this disease is endemic. Miltefosine is an effective but expensive option for systemic therapy; its oral dosing is currently FDA-approved in the United States for treating leishmaniasis.^{30,68-71} Pentamidine is a little-used therapy owing to its side effects and limited data on its efficacy.⁷² Azoles such as ketoconazole, fluconazole, itraconazole, and posaconazole are alternative therapies, but ones that show variable effectiveness compared to other options based on the particular species of *Leishmania* parasite involved.^{60,73-79} The use and early promise of high-dose fluconazole for treatment against *L. (V.) braziliensis*⁸⁰ is no longer considered an option based on the sub-optimal results from a clinical trial, thus illustrating the need for further clinical trials of other medicines and the need for early-stage research for discovering future treatment options.⁸¹ The complexity of diagnosis and treatment of the various species of *Leishmania* is outlined in practice guidelines issued by a joint working group of The Infectious Diseases Society of America and the American Society of Tropical Medicine and Hygiene.^{82,83}

Prevention and Control

Since humans are often dead-end hosts, treating infected humans is not expected to impact disease transmission significantly.⁸⁴ Although dogs have been identified as a significant reservoir, infection in multiple wild

and domesticated mammals is not observed.⁸⁵ Eradication of sand fly breeding sites near suburban and urban centers, attaching pyrethroid-impregnated collars to domestic dogs, and sleeping under insecticide-impregnated bed netting are cost-effective control measures.⁶⁰ Sand flies only bite at certain times of the day in a crepuscular/nocturnal pattern: in the morning, late in the evening, and at night.⁸⁶ Although avoidance of outdoor activities during these times reduces the chance of being bitten, it can be difficult to implement that advice for travelers to endemic areas, who may have limited time to accomplish what they have on their itinerary and perhaps impossible for long-term residents who do not have the luxury to adjust their

schedule. Additional, and possibly more viable, approaches to avoid sand fly bites in high-risk areas are using insect repellents and insecticide-treated clothing that covers most of the body.^{87, 88}

Vaccines employing combinations of parasite antigens offer hope that a standard vaccine for use against CL and VL may eventually be available, but no human vaccine is currently available.^{18, 89} A CL vaccine could be economically viable based on cost and efficacy.⁹⁰ First-generation vaccines using whole-killed or purified fractions, genetically modified attenuated vaccines, DNA vaccines, and sand fly saliva vaccines are all being explored.⁹¹

References

1. Azami-Conesa, I., et al., A Systematic Review (1990-2021) of Wild Animals Infected with Zoonotic Leishmania. *Microorganisms* **2021**, 9 (5).
2. Berman, J., Recent Developments in Leishmaniasis: Epidemiology, Diagnosis, and Treatment. *Current infectious disease reports* **2005**, 7 (1), 33-38.
3. Magill, A. J., et al., Visceral infection caused by *Leishmania tropica* in veterans of Operation Desert Storm. *N Engl J Med* **1993**, 328 (19), 1383-7.
4. Alvar, J., et al., Leishmaniasis worldwide and global estimates of its incidence. *PLoS One* **2012**, 7 (5), e35671.
5. Rassi, Y., et al., Vectors and reservoirs of cutaneous leishmaniasis in Marvdasht district, southern Islamic Republic of Iran. *East Mediterr Health J* **2007**, 13 (3), 686-93.
6. Hotez, P. J., Vaccine Science Diplomacy: Expanding Capacity to Prevent Emerging and Neglected Tropical Diseases Arising from Islamic State (IS)--Held Territories. *PLoS Negl Trop Dis* **2015**, 9 (9), e0003852.
7. Beyrer, C., et al., Neglected diseases, civil conflicts, and the right to health. *Lancet* **2007**, 370 (9587), 619-27.
8. Barry, M. A., et al., Cutaneous leishmaniasis in Cuban immigrants to Texas who traveled through the Darien Jungle, Panama. *Am J Trop Med Hyg* **2014**, 91 (2), 345-7.
9. Russell, A.; Millar, A., Natural History of Aleppo and Parts Adjacent. 1856; p 262-266.
10. Theodorides, J., Note historique sur la decouverte de la transmission de la leishmaniose cutanee par les phlebotomes. *Bull Soc Pathol Exot* **1997**, 90, 177-180.
11. Cunningham, D. D., On the presence of peculiar parasitic organisms in the tissue of a specimen of Delhi boil. *Sci Mem Med Officers Army India* **1885**, 1 21-31.
12. Handman, E., Leishmaniasis: current status of vaccine development. *Clinical microbiology reviews* **2001**, 14 (2), 229-43.
13. McCall, L. I., et al., Leishmanization revisited: immunization with a naturally attenuated cutaneous *Leishmania donovani* isolate from Sri Lanka protects against visceral leishmaniasis. *Vaccine* **2013**, 31 (10), 1420-5.
14. Greenblatt, C. L., The present and future of vaccination for cutaneous leishmaniasis. *Prog Clin Biol Res* **1980**, 47, 259-85.
15. del Giudice, P., et al., Cutaneous leishmaniasis due to *Leishmania infantum*. Case reports and literature review. *Archives of dermatology* **1998**, 134 (2), 193-8.

16. Bates, P. A., Transmission of *Leishmania* metacyclic promastigotes by Phlebotomine sand flies. *Int J Parasitol* **2007**, 37 (10), 1097-106.
17. Lima, H. C., et al., Resolution of an infection with *Leishmania braziliensis* confers complete protection to a subsequent challenge with *Leishmania major* in BALB/c mice. *Memorias do Instituto Oswaldo Cruz* **1999**, 94 (1), 71-6.
18. Coler, R. N.; Reed, S. G., Second-generation vaccines against leishmaniasis. *Trends in parasitology* **2005**, 21 (5), 244-9.
19. Mansueto, P., et al., Leishmaniasis in travelers: a literature review. *Travel Med Infect Dis* **2014**, 12 (6 Pt A), 563-81.
20. Lainson, R., et al., New World Leishmaniasis-The Neotropical *Leishmania* Species. 2005.
21. van Griensven, J., et al., Leishmaniasis in immunosuppressed individuals. *Clin Microbiol Infect* **2014**, 20 (4), 286-99.
22. Lianon, R.; Strangeways-Dixon, J., *Leishmania mexicana*: The epidemiology of dermal Leishmaniasis in British Honduras. *Trans Roy Soc Trop Med Hyg* **1963**, 57, 242-265.
23. Hailu, A., The use of direct agglutination test (DAT) in serological diagnosis of Ethiopian cutaneous leishmaniasis. *Diagnostic microbiology and infectious disease* **2002**, 42 (4), 251-6.
24. Akuffo, H., et al., *Leishmania aethiopica* derived from diffuse leishmaniasis patients preferentially induce mRNA for interleukin-10 while those from localized leishmaniasis patients induce interferon-gamma. *The Journal of infectious diseases* **1997**, 175 (3), 737-41.
25. Bryceson, A. D., Diffuse cutaneous leishmaniasis in Ethiopia. I. The clinical and histological features of the disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1969**, 63 (6), 708-37.
26. Turetz, M. L., et al., Disseminated leishmaniasis: a new and emerging form of leishmaniasis observed in northeastern Brazil. *The Journal of infectious diseases* **2002**, 186 (12), 1829-34.
27. Dassoni, F., et al., Cutaneous and mucocutaneous leishmaniasis resembling borderline-tubercloid leprosy: a new clinical presentation? *Acta Derm Venereol* **2013**, 93 (1), 74-7.
28. Bonfante-Garrido, R., et al., Disseminated American cutaneous leishmaniasis. *International journal of dermatology* **1996**, 35 (8), 561-5.
29. Sharquie, K. E.; Najim, R. A., Disseminated cutaneous leishmaniasis. *Saudi medical journal* **2004**, 25 (7), 951-4.
30. Machado, P. R.; Penna, G., Miltefosine and cutaneous leishmaniasis. *Curr Opin Infect Dis* **2012**, 25 (2), 141-4.
31. Ordaz-Farias, A., et al., Case report: Transient success using prolonged treatment with miltefosine for a patient with diffuse cutaneous leishmaniasis infected with *Leishmania mexicana mexicana*. *Am J Trop Med Hyg* **2013**, 88 (1), 153-6.
32. Postigo, C., et al., Cutaneous lesions in patients with visceral leishmaniasis and HIV infection. *The Journal of infection* **1997**, 35 (3), 265-8.
33. Handler, M. Z., et al., Cutaneous and mucocutaneous leishmaniasis: Differential diagnosis, diagnosis, histopathology, and management. *J Am Acad Dermatol* **2015**, 73 (6), 911-26.
34. Boggild, A. K., et al., Diagnostic performance of filter paper lesion impression PCR for secondarily infected ulcers and nonulcerative lesions caused by cutaneous leishmaniasis. *J Clin Microbiol* **2011**, 49 (3), 1097-100.
35. Boggild, A. K., et al., Detection and species identification of *Leishmania* DNA from filter paper lesion impressions for patients with American cutaneous leishmaniasis. *Clin Infect Dis* **2010**, 50 (1), e1-6.
36. Boggild, A. K., et al., Non-invasive cytology brush PCR diagnostic testing in mucosal leishmaniasis: superior performance to conventional biopsy with histopathology. *PloS one* **2011**, 6 (10), e26395.
37. Mimori, T., et al., Rapid identification of *Leishmania* species from formalin-fixed biopsy samples by polymorphism-specific polymerase chain reaction. *Gene* **1998**, 210 (2), 179-86.
38. Gangneux, J.-P., et al., Prospective value of PCR amplification and sequencing for diagnosis and typing of old world *Leishmania* infections in an area of nonendemicity. *Journal of clinical microbiology* **2003**, 41 (4), 1419-22.

39. Mouttaki, T., et al., Molecular diagnosis of cutaneous leishmaniasis and identification of the causative *Leishmania* species in Morocco by using three PCR-based assays. *Parasit Vectors* **2014**, 7, 420.
40. Nzelu, C. O., et al., A rapid molecular diagnosis of cutaneous leishmaniasis by colorimetric malachite green-loop-mediated isothermal amplification (LAMP) combined with an FTA card as a direct sampling tool. *Acta Trop* **2016**, 153, 116-9.
41. Yehia, L., et al., Molecular diagnosis of cutaneous leishmaniasis and species identification: analysis of 122 biopsies with varied parasite index. *J Cutan Pathol* **2012**, 39 (3), 347-55.
42. Palumbo, E., Treatment strategies for mucocutaneous leishmaniasis. *J Glob Infect Dis* **2010**, 2 (2), 147-50.
43. Faber, W. R., et al., Value of diagnostic techniques for cutaneous leishmaniasis. *Journal of the American Academy of Dermatology* **2003**, 49 (1), 70-4.
44. Dey, T., et al., Infectivity and virulence of *Leishmania donovani* promastigotes: a role for media, source, and strain of parasite. *The Journal of eukaryotic microbiology* **2002**, 49 (4), 270-4.
45. Luz, Z. M., et al., Lesion aspirate culture for the diagnosis and isolation of *Leishmania* spp. from patients with cutaneous leishmaniasis. *Mem Inst Oswaldo Cruz* **2009**, 104 (1), 62-6.
46. Castelli, G., et al., Evaluation of two modified culture media for *Leishmania infantum* cultivation versus different culture media. *J Parasitol* **2014**, 100 (2), 228-30.
47. Szargiki, R., et al., Comparison of serological and parasitological methods for cutaneous leishmaniasis diagnosis in the state of Parana, Brazil. *Braz J Infect Dis* **2009**, 13 (1), 47-52.
48. Skraba, C. M., et al., Evaluation of the reference value for the Montenegro skin test. *Rev Soc Bras Med Trop* **2015**, 48 (4), 437-44.
49. Turgay, N., et al., Quantiferon-*Leishmania* as an epidemiological tool for evaluating the exposure to *Leishmania* infection. *Am J Trop Med Hyg* **2010**, 83 (4), 822-4.
50. Alimohammadian, M. H., et al., Assessment of interferon-gamma levels and leishmanin skin test results in persons recovered for leishmaniasis. *Am J Trop Med Hyg* **2012**, 87 (1), 70-5.
51. Bogdan, C., et al., Invasion, control and persistence of *Leishmania* parasites. *Curr Opin Immunol* **1996**, 8 (4), 517-25.
52. Mendonca, M. G., et al., Persistence of *Leishmania* parasites in scars after clinical cure of American cutaneous leishmaniasis: is there a sterile cure? *J Infect Dis* **2004**, 189 (6), 1018-23.
53. Willard, R. J., et al., Cutaneous leishmaniasis in soldiers from Fort Campbell, Kentucky returning from Operation Iraqi Freedom highlights diagnostic and therapeutic options. *J Am Acad Dermatol* **2005**, 52 (6), 977-87.
54. Morizot, G., et al., Travelers with cutaneous leishmaniasis cured without systemic therapy. *Clin Infect Dis* **2013**, 57 (3), 370-80.
55. al-Majali, O., et al., A 2-year study of liquid nitrogen therapy in cutaneous leishmaniasis. *Int J Dermatol* **1997**, 36 (6), 460-2.
56. Dobrev, H. P., et al., Cutaneous Leishmaniasis - Dermoscopic Findings And Cryotherapy. *Folia Med (Plovdiv)* **2015**, 57 (1), 65-8.
57. Farajzadeh, S., et al., Comparison between Combination Therapy of Oral Terbinafine and Cryotherapy versus Systemic Meglumine Antimoniate and Cryotherapy in Cutaneous Leishmaniasis: A Randomized Clinical Trial. *Iran J Parasitol* **2015**, 10 (1), 1-8.
58. Pizinger, K., et al., Successful treatment of cutaneous leishmaniasis by photodynamic therapy and cryotherapy. *Eur J Dermatol* **2009**, 19 (2), 172-3.
59. Sbeghen, M. R., et al., Topical and intradermal efficacy of photodynamic therapy with methylene blue and light-emitting diode in the treatment of cutaneous leishmaniasis caused by *Leishmania braziliensis*. *J Lasers Med Sci* **2015**, 6 (3), 106-11.
60. Shazad, B., et al., Comparison of topical paromomycin sulfate (twice/day) with intralesional meglumine antimoniate for the treatment of cutaneous leishmaniasis caused by *L. major*. *European journal of dermatology : EJD* **2005**, 15 (2), 85-7.
61. Soto, J., et al., Intralesional antimony for single lesions of bolivian cutaneous leishmaniasis. *Clin Infect Dis* **2013**, 56 (9), 1255-60.
62. Shin, J. Y., et al., New world cutaneous leishmaniasis treated with intralesional injection of pentavalent antimony. *Ann Dermatol* **2013**, 25 (1), 80-3.

63. Singh, N., et al., Leishmaniasis: current status of available drugs and new potential drug targets. *Asian Pac J Trop Med* **2012**, 5 (6), 485-97.
64. Hodiamont, C. J., et al., Species-directed therapy for leishmaniasis in returning travellers: a comprehensive guide. *PLoS Negl Trop Dis* **2014**, 8 (5), e2832.
65. Steverding, D., The history of leishmaniasis. *Parasit Vectors* **2017**, 10 (1), 82.
66. Aronson, N. E., et al., Safety and efficacy of intravenous sodium stibogluconate in the treatment of leishmaniasis: recent U.S. military experience. *Clin Infect Dis* **1998**, 27 (6), 1457-64.
67. Sundar, S.; Chakravarty, J., Liposomal amphotericin B and leishmaniasis: dose and response. *J Glob Infect Dis* **2010**, 2 (2), 159-66.
68. Madke, B., et al., Successful treatment of multifocal cutaneous leishmaniasis with miltefosine. *Indian J Dermatol* **2011**, 56 (5), 587-90.
69. Rubiano, L. C., et al., Noninferiority of miltefosine versus meglumine antimoniate for cutaneous leishmaniasis in children. *J Infect Dis* **2012**, 205 (4), 684-92.
70. Monge-Maillo, B.; Lopez-Velez, R., Miltefosine for visceral and cutaneous leishmaniasis: drug characteristics and evidence-based treatment recommendations. *Clin Infect Dis* **2015**, 60 (9), 1398-404.
71. Dorlo, T. P., et al., Dynamics of parasite clearance in cutaneous leishmaniasis patients treated with miltefosine. *PLoS Negl Trop Dis* **2011**, 5 (12), e1436.
72. Jaureguierry, S., et al., Efficacy of short-course intramuscular pentamidine isethionate treatment on Old World localized cutaneous leishmaniasis in 2 patients. *Clin Infect Dis* **2006**, 42 (12), 1812-3.
73. Laffitte, E., et al., Cutaneous leishmaniasis caused by *Leishmania tropica*: treatment with oral fluconazole. *Dermatology (Basel, Switzerland)* **2005**, 210 (3), 249-51.
74. Morizot, G., et al., Healing of Old World cutaneous leishmaniasis in travelers treated with fluconazole: drug effect or spontaneous evolution? *Am J Trop Med Hyg* **2007**, 76 (1), 48-52.
75. Saenz, R. E., et al., Efficacy of ketoconazole against *Leishmania braziliensis* panamensis cutaneous leishmaniasis. *Am J Med* **1990**, 89 (2), 147-55.
76. Firooz, A., et al., Itraconazole in the treatment of cutaneous leishmaniasis. *Int J Dermatol* **2006**, 45 (12), 1446-7.
77. Baroni, A., et al., Cutaneous leishmaniasis treated with itraconazole. *Dermatol Ther* **2009**, 22 Suppl 1, S27-9.
78. Saleem, K.; Rahman, A., Comparison of oral itraconazole and intramuscular meglumine antimoniate in the treatment of cutaneous leishmaniasis. *J Coll Physicians Surg Pak* **2007**, 17 (12), 713-6.
79. Paniz Mondolfi, A. E., et al., Successful treatment of Old World cutaneous leishmaniasis caused by *Leishmania infantum* with posaconazole. *Antimicrob Agents Chemother* **2011**, 55 (4), 1774-6.
80. Sousa, A. Q., et al., High-dose oral fluconazole therapy effective for cutaneous leishmaniasis due to *Leishmania (Vianna) braziliensis*. *Clin Infect Dis* **2011**, 53 (7), 693-5.
81. Corman, H. N., et al., Drug Discovery for Cutaneous Leishmaniasis: A Review of Developments in the Past 15 Years. *Microorganisms* **2023**, 11 (12).
82. Prates, F. V., et al., Fluconazole in the Treatment of Cutaneous Leishmaniasis Caused by *Leishmania braziliensis*: A Randomized Controlled Trial. *Clin Infect Dis* **2017**, 64 (1), 67-71.
83. Aronson, N., et al., Diagnosis and Treatment of Leishmaniasis: Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). *Am J Trop Med Hyg* **2017**, 96 (1), 24-45.
84. Maroli, M.; Khoury, C., [Prevention and control of leishmaniasis vectors: current approaches]. *Parassitologia* **2004**, 46 (1-2), 211-5.
85. Quinnell, R. J.; Courtenay, O., Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitology* **2009**, 136 (14), 1915-34.
86. Rivas, G. B., et al., Effects of temperature and photoperiod on daily activity rhythms of *Lutzomyia longipalpis* (Diptera: Psychodidae). *Parasit Vectors* **2014**, 7, 278.
87. Banks, S. D., et al., Insecticide-treated clothes for the control of vector-borne diseases: a review on effectiveness and safety. *Med Vet Entomol* **2014**, 28 Suppl 1, 14-25.

88. Moore, S. J., et al., Insect bite prevention. *Infect Dis Clin North Am* **2012**, *26* (3), 655-73.
89. Kumar, R.; Engwerda, C., Vaccines to prevent leishmaniasis. *Clin Transl Immunology* **2014**, *3* (3), e13.
90. Bacon, K. M., et al., The potential economic value of a cutaneous leishmaniasis vaccine in seven endemic countries in the Americas. *Vaccine* **2013**, *31* (3), 480-6.
91. Dinc, R., Leishmania Vaccines: the Current Situation with Its Promising Aspect for the Future. *Korean J Parasitol* **2022**, *60* (6), 379-391.

4. Mucocutaneous Leishmaniasis

Pronunciation: \lēsh-'ma-nē-ə\

Leishmania Viannia braziliensis
(Vianna 1911)

Leishmania Viannia guyanensis
(Floch 1954)

Leishmania Viannia panamensis
(Lainson & Shaw 1972)

Leishmania Viannia peruviana

Introduction

There are currently approximately twenty species of *Leishmania* (LEESH-ma-NEE-ah\) that are capable of causing mucocutaneous leishmaniasis (MCL; also known as espundia), with the majority of subspecies classified in the *L. Viannia* subgenus.¹ *L. (V.) braziliensis* is mainly responsible for MCL, perhaps due to its ability to trigger a T-cell hypersensitivity response.² *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis*, *L. (V.) peruviana*, and other members of the *Viannia* subgenus, including hybrid species, can cause mucocutaneous disease.³ It is estimated

that without treatment, the different rates of mucocutaneous disease for the different subspecies are 10-30% for *L. (V.) braziliensis*, 10% for *L. (V.) guyanensis*, less than 10% for *L. (V.) panamensis*, and rarely for other members of the *Viannia* subgenus. While mucocutaneous disease has been observed to develop at different frequencies in patients initially infected with these subspecies, the risk appears to depend not only on subspecies but also on a patient's gender, age, and nutritional status.⁴ Usually following primary infection or rarely coincident with cutaneous disease, the parasites metastasize to mucocutaneous junctions (oral cavity, nares, and anal areas), where they erode the soft tissues.⁵ This is often a disfiguring condition as it can affect the face and lead to extensive destruction.^{6,7} Since MCL is generally restricted to the New World, sandflies of the genus *Lutzomyia* are considered the vector.⁸ MCL is mainly concentrated in Ecuador, Bolivia, Peru, and Northern Brazil, the so-called mucocutaneous belt.⁹ A number of the clinical cases of MCL involve *Leishmania* parasites infected with the double-stranded RNA virus, *Leishmania* RNA Virus (LRV), which appears to correlate with disease severity and relapse due to treatment failure.¹⁰⁻¹²



Figure 4.1. Destruction from cutaneous lesion on lower lip due to *L. braziliensis*.

Historical Information

In 1911, Antonio Carini identified patients suffering from mucocutaneous lesions as different from those only demonstrating cutaneous lesions, thereby establishing MCL as a separate clinical entity.¹³ The following year, in 1912, Gaspar Vianna identified and named *L. (V.) braziliensis* and succeeded in characterizing this parasite as a distinct species despite the lack of modern laboratory equipment using only serology and culture.^{14,15}

Life Cycle

The disease is initiated when an infected sand fly takes a blood meal, as with cutane-

Leishmania braziliensis

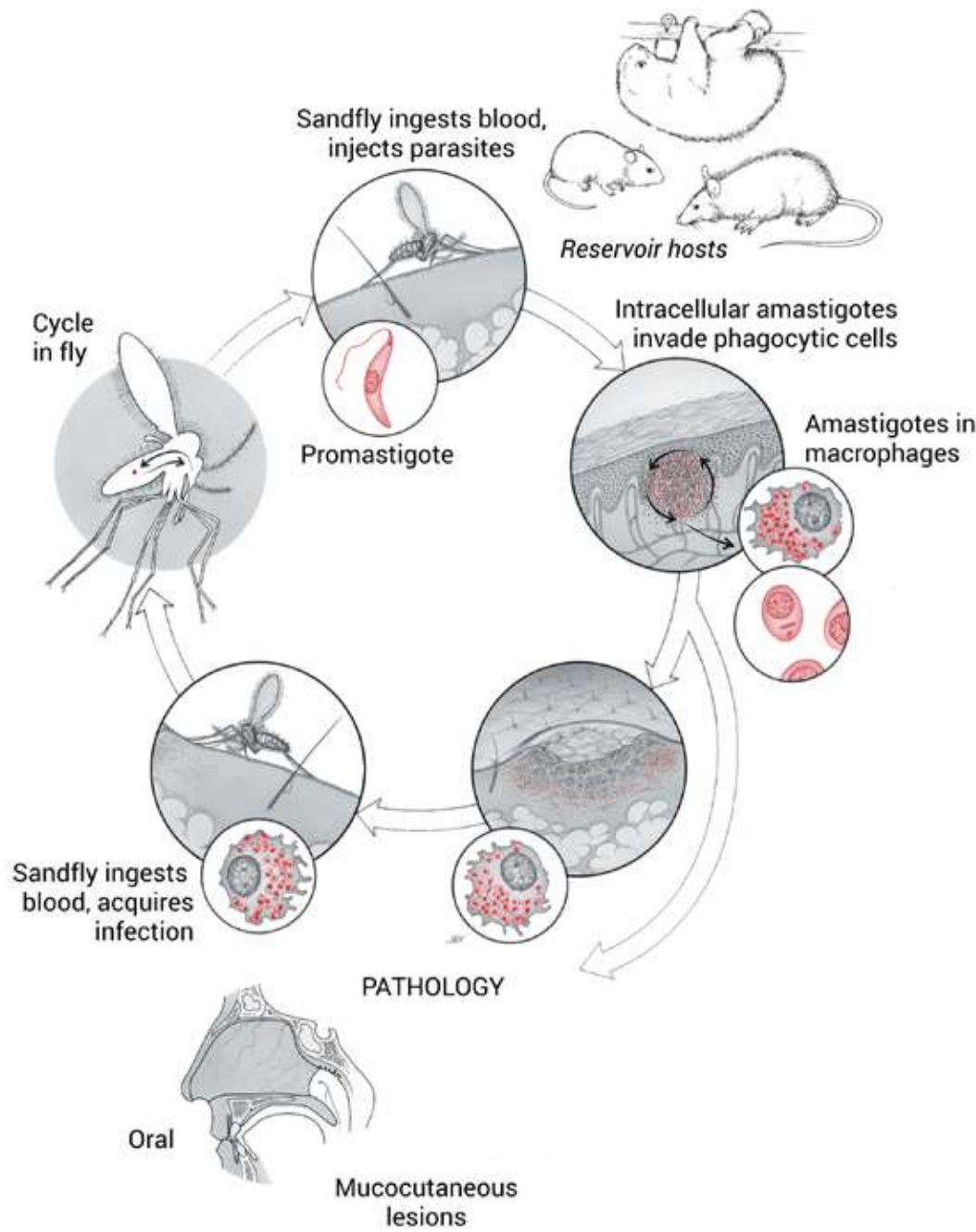




Figure 4.2. Espundia. This lesion resulted in the erosion of the soft palate. Most lesions do not advance this far before medical intervention.

ous leishmaniasis (CL). Metacyclic promastigotes are injected into the subcutaneous tissue and adhere to the extracellular matrix. A primary lesion forms at the bite site as the result of infection of dendritic cells and macrophages (Fig. 2.3). The lesion evolves into an ulcer (Fig. 4.1), which is indistinguishable from that induced by any number of other species causing CL. Amastigotes are transported to the mucocutaneous junction early in infection, although lesions at this site are slow to appear, even if the patient develops MCL.⁵ At these distant mucocutaneous sites, the amastigotes divide within resident macrophages, and tissue erosion develops. This destruction is likely attributable more to an exuberant host response driven by an exaggerated T-cell response than any factors intrinsic to the parasite.² Perhaps the presence of LRV infecting the responsible *Leishmania* parasites plays a role in augmenting this immune response and driving the hyper-

sensitivity response.^{12, 16-18} Infections typically “smolder” for long periods, usually months to years.

Person-to-person transmission has not been observed. This is probably a zoonotic species, infecting reservoir hosts (domestic dogs, rodents, and various rainforest mammals).¹⁹

Clinical Disease

MCL lesions developing in the nasal passage (the most common mucocutaneous site) are characterized by necrotizing inflammation of mixed cell types (plasma cells and lymphocytes).⁶ Skin, mucous membranes, and cartilage can also be involved.²⁰ Lesions of the oral cavity can destroy the soft palate, nasal septum, and even the larynx.²¹ When this occurs, the patient may die from an infection that spreads to the lungs.⁵ MCL is a pauciparasitic disease, and parasites are seldom demonstrated in lesions, although PCR can reveal them.²² The ulcers are replaced by fibrous granulomas that heal slowly, scar, and deform the tissues. The most advanced cases are known as “espundia” (Fig. 4.2).²³ Unlike CL, MCL does not usually heal spontaneously.^{9, 20}

Multiple cutaneous lesions and extensive mucocutaneous involvement have been described in patients co-infected with HIV-1.²⁴⁻²⁶ Due to enormous genetic variation among strains of *Leishmania* spp. causing MCL, the disease patterns in rarely occurring MCL infections in HIV-1 patients present an exceedingly complex and challenging clinical picture.^{27, 28}

Diagnosis (see Clinical Appendix)

Definitive diagnosis and subspecies identification is by NAAT.²⁹ Organisms are difficult to find in the biopsy of infected palate

and soft tissues of the oral cavity due to the pauci-microbial nature of these infections, so microscopy of histological sections is not a great option for diagnosis. Culture of biopsy material is a possibility (see diagnosis for CL), but the growth of promastigotes at room temperature is slow and only available in certain centers.

Treatment (see Clinical Appendix)

As a general rule, systemic therapies are used to treat MCL, including pentavalent antimony, amphotericin B, miltefosine, pentamidine, or azole drugs.^{9,30} Sodium stibogluconate, an antimony-containing drug with many serious side effects, including rash, headache, arthralgias and myalgias, pancreatitis, transaminitis, and hematologic suppression, is commonly used in many parts of the world, in part due to its relatively low cost.³¹ Amphotericin B and its liposomal form are highly effective options for treatment, and many now consider the liposomal form the drug of choice for MCL and visceral leishmaniasis (VL). In the developing world, standard amphotericin is often employed to treat MCL if antimonial therapy fails. Miltefosine is an effective but expensive option for systemic therapy with oral dosing currently FDA-approved in the United States for treating leishmaniasis.³²⁻³⁶ Pentamidine is another option that has demonstrated efficacy in treating MCL.^{37, 38} Azoles such as ketoconazole, fluconazole, itraconazole, and posaconazole are alternative oral therapies with variable efficacy compared to other options based on the particular species of *Leishmania* parasite involved.³⁹⁻⁴⁶ Given the complexity of issues and incomplete information the medications, Guidelines from tropical medicine experts should be consulted such as the recommendations from the joint working group of The Infectious Diseases Society of America and the American Society of Tropical Medicine and Hygiene.⁴⁷

None of the above therapies eliminates all parasites in all patients. Persistence of infection beyond the treatment period has been recorded in longstanding infections, as demonstrated by PCR on experimentally infected monkeys and in follow-up of some patients.⁴⁸

Prevention and Control

Transmission of *L. (V.) braziliensis* and related species capable of causing MCL (*L. (V.) guyanensis*, *L. (V.) panamensis*, and *L. (V.) peruviana* occur through close contact of human populations with reservoir hosts (wild and domestic mammals). Deforestation of vast rainforest regions for agriculture, mining, and oil exploration, which leads to increased urbanization in South and Central America, has exposed many people to rainforest edges. Edges of natural systems are particular ecologically defined areas termed ecotones.^{37, 49} These zones constitute the borders between ecosystems and are further characterized by ecological stress on all plant and animal species in that zone due to intra and inter-specific competition for resources. Moreover, altitudes of approximately 2000 meters or more have been designated as areas of increased risk for MCL.⁴⁷

The distribution of sand fly species is determined by the availability of natural habitats and that created by human encroachment. Some species favor unaltered forest, while others thrive in ecologically disturbed situations.³⁷ Still, many could be given that would reinforce the idea that when ecological change due to human settlement occurs, those living there are at greater risk of acquiring zoonotic infectious diseases (e.g., Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus).⁴⁹ The number of species of *Leishmania* known to infect humans throughout South America alone has increased dramatically.⁵

Avoiding contact with vectors of MCL is an ineffective recommendation, given the need for more awareness of their presence on the part of those living near them. The following advice may be helpful for those traveling to endemic areas wishing to avoid the bites of sandflies. Sand flies are more prevalent during the rainy season.⁵⁰ Sand flies only bite at certain times of the day in a crepuscular/nocturnal pattern: in the morning, late in the evening, and at night.⁸ Although avoidance of outdoor activities during these times reduces the chance of being bitten, this can be difficult advice for travelers to endemic areas, who may have limited time to accomplish what they have on their itinerary and perhaps impossible for long-term residents who do not have the luxury to adjust their schedule. Additional and more viable approaches to avoid sand fly bites used by travelers and workers in high-risk areas are using insect repellents and insecticide-treated clothing that covers most of the body.^{51, 52} Growing

rates of insecticide resistance in sand fly populations are already limiting the utility of this approach in many parts of the world.

Using insecticide-impregnated clothing and bed netting has helped reduce disease transmission for malaria, particularly in Africa. It has now been proven effective in reducing the transmission of *Leishmania* as well. Maintaining wide buffer zones between settlements and the surrounding native forest appears to be the best environmentally based long-term solution to controlling the incidence of infection in endemic transmission zones, a recommendation made initially to those wishing to avoid becoming infected with the Yellow fever virus. However, curtailing this human imperative has, so far, been all but impossible to enforce.

Vaccines using parasite antigens offer hope, but no human vaccine is available despite continued efforts.⁵³⁻⁵⁵

References

1. Herwaldt, B. L., Leishmaniasis. *Lancet (London, England)* **1999**, 354 (9185), 1191-9.
2. Silveira, F. T., et al., Immunopathogenic competences of *Leishmania (V.) braziliensis* and *L. (L.) amazonensis* in American cutaneous leishmaniasis. *Parasite Immunol* **2009**, 31 (8), 423-31.
3. Steverding, D., The history of leishmaniasis. *Parasit Vectors* **2017**, 10 (1), 82.
4. Machado-Coelho, G. L., et al., Risk factors for mucosal manifestation of American cutaneous leishmaniasis. *Trans R Soc Trop Med Hyg* **2005**, 99 (1), 55-61.
5. Lainson, R., et al., New World Leishmaniasis-The Neotropical *Leishmania* Species. 2005.
6. Crovetto-Martinez, R., et al., Mucocutaneous leishmaniasis must be included in the differential diagnosis of midline destructive disease: two case reports. *Oral Surg Oral Med Oral Pathol Oral Radiol* **2015**, 119 (1), e20-6.
7. Garcia, A. L., et al., American tegumentary leishmaniasis: antigen-gene polymorphism, taxonomy and clinical pleomorphism. *Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases* **2005**, 5 (2), 109-16.
8. Rivas, G. B., et al., Effects of temperature and photoperiod on daily activity rhythms of *Lutzomyia longipalpis* (Diptera: Psychodidae). *Parasit Vectors* **2014**, 7, 278.
9. Palumbo, E., Treatment strategies for mucocutaneous leishmaniasis. *J Glob Infect Dis* **2010**, 2 (2), 147-50.
10. Chung, I. K., et al., Generation of the short RNA transcript in *Leishmanivirus* correlates with the growth of its parasite host, *Leishmania*. *Molecules and cells* **1998**, 8 (1), 54-61.
11. Ogg, M. M., et al., Short report: quantification of leishmanivirus RNA in clinical samples and its possible role in pathogenesis. *The American journal of tropical medicine and hygiene* **2003**, 69 (3),

- 309-13.
12. Ives, A., et al., Leishmania RNA virus controls the severity of mucocutaneous leishmaniasis. *Science* **2011**, 331 (6018), 775-8.
13. Carini, A., Leishmaniose de Ia muqucuse rhinobuccopharyngee. *Bull Soc Pathol Exot* **1911**, 4 289-291.
14. Kligler, I. J., Some Regulating Factors in Bacterial Metabolism. *Journal of bacteriology* **1916**, 1 (6), 663-71.
15. Noguchi, H.; S., Comparative studies on herpetomonads and Leishmaniasis. *II Differentiation of the organisms by serological reactions and fermentation tests Med* **1929**, 44, 305-314.
16. Kariyawasam, R., et al., Novel detection of Leishmania RNA virus-1 (LRV-1) in clinical isolates of Leishmania Viannia panamensis. *Parasitology* **2023**, 1-22.
17. Kariyawasam, R., et al., Leishmania RNA Virus 1 (LRV-1) in Leishmania (Viannia) braziliensis Isolates from Peru: A Description of Demographic and Clinical Correlates. *Am J Trop Med Hyg* **2020**, 102 (2), 280-285.
18. Kariyawasam, R., et al., Influence of Leishmania RNA Virus 1 on Proinflammatory Biomarker Expression in a Human Macrophage Model of American Tegumentary Leishmaniasis. *J Infect Dis* **2017**, 216 (7), 877-886.
19. Alexander, B., et al., Detection of *Leishmania (Viannia) braziliensis* complex in wild mammals from Colombian coffee plantations by PCR and DNA hybridization. *Acta tropica* **1998**, 69 (1), 41-50.
20. Ahluwalia, S., et al., Mucocutaneous leishmaniasis: an imported infection among travellers to central and South America. *Bmj* **2004**, 329 (7470), 842-4.
21. Lohuis, P. J., et al., *Leishmania braziliensis* presenting as a granulomatous lesion of the nasal septum mucosa. *The Journal of laryngology and otology* **1997**, 111 (10), 973-5.
22. Belli, A., et al., Simplified polymerase chain reaction detection of new world *Leishmania* in clinical specimens of cutaneous leishmaniasis. *The American journal of tropical medicine and hygiene* **1998**, 58 (1), 102-9.
23. Marsden, P. D., Mucosal leishmaniasis ("espundia" Escomel, 1911). *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1986**, 80 (6), 859-76.
24. Machado, E. S., et al., Disseminated American muco-cutaneous leishmaniasis caused by *Leishmania braziliensis braziliensis* in a patient with AIDS: a case report. *Memorias do Instituto Oswaldo Cruz* **1992**, 87 (4), 487-92.
25. Mattos, M., et al., American cutaneous leishmaniasis associated with HIV infection: report of four cases. *Journal of the European Academy of Dermatology and Venereology : JEADV* **1998**, 10 (3), 218-25.
26. Torrico, F., et al., Co-Infection of *Leishmania (Viannia) braziliensis* and HIV: report of a case of mucosal leishmaniasis in Cochabamba, Bolivia. *Am J Trop Med Hyg* **2009**, 81 (4), 555-8.
27. Da-Cruz, A. M., et al., Atypical mucocutaneous leishmaniasis caused by *Leishmania braziliensis* in an acquired immunodeficiency syndrome patient: T-cell responses and remission of lesions associated with antigen immunotherapy. *Memorias do Instituto Oswaldo Cruz* **1999**, 94 (4), 537-42.
28. Chouicha, N.; Lanotte, G., Phylogenetic taxonomy of *Leishmania (viannia) braziliensis* based on isoenzymatic study of 137 isolates. *Parasitology* **1998**, 115, 343-8.
29. Olivier, M., et al., Subversion mechanisms by which *Leishmania* parasites can escape the host immune response: a signaling point of view. *Clinical microbiology reviews* **2005**, 18 (2), 293-305.
30. Singh, N., et al., Leishmaniasis: current status of available drugs and new potential drug targets. *Asian Pac J Trop Med* **2012**, 5 (6), 485-97.
31. Aronson, N. E., et al., Safety and efficacy of intravenous sodium stibogluconate in the treatment of leishmaniasis: recent U.S. military experience. *Clin Inf Dis* **1998**, 27 (6), 1457-64.
32. Madke, B., et al., Successful treatment of multifocal cutaneous leishmaniasis with miltefosine. *Indian J Dermatol* **2011**, 56 (5), 587-90.
33. Rubiano, L. C., et al., Noninferiority of miltefosine versus meglumine antimoniate for cutaneous leishmaniasis in children. *J Infect Dis* **2012**, 205 (4), 684-92.
34. Monge-Maillo, B.; Lopez-Velez, R., Miltefosine for visceral and cutaneous leishmaniasis: drug

- characteristics and evidence-based treatment recommendations. *Clin Infect Dis* **2015**, 60 (9), 1398-404.
35. Machado, P. R.; Penna, G., Miltefosine and cutaneous leishmaniasis. *Curr Opin Infect Dis* **2012**, 25 (2), 141-4.
 36. Dorlo, T. P., et al., Dynamics of parasite clearance in cutaneous leishmaniasis patients treated with miltefosine. *PLoS Negl Trop Dis* **2011**, 5 (12), e1436.
 37. Salomon, O. D., et al., Ecological aspects of phlebotomine (Diptera, Psychodidae) in an endemic area of tegumentary leishmaniasis in the northeastern Argentina, 1993-1998. *Memorias do Instituto Oswaldo Cruz* **2002**, 97 (2), 163-8.
 38. Jaureguiberry, S., et al., Efficacy of short-course intramuscular pentamidine isethionate treatment on Old World localized cutaneous leishmaniasis in 2 patients. *Clin Infect Dis* **2006**, 42 (12), 1812-3.
 39. Laffitte, E., et al., Cutaneous leishmaniasis caused by *Leishmania tropica*: treatment with oral fluconazole. *Dermatology (Basel, Switzerland)* **2005**, 210 (3), 249-51.
 40. Shazad, B., et al., Comparison of topical paromomycin sulfate (twice/day) with intralesional meglumine antimoniate for the treatment of cutaneous leishmaniasis caused by *L. major*. *European journal of dermatology : EJD* **2005**, 15 (2), 85-7.
 41. Morizot, G., et al., Healing of Old World cutaneous leishmaniasis in travelers treated with fluconazole: drug effect or spontaneous evolution? *Am J Trop Med Hyg* **2007**, 76 (1), 48-52.
 42. Saenz, R. E., et al., Efficacy of ketoconazole against *Leishmania braziliensis panamensis* cutaneous leishmaniasis. *Am J Med* **1990**, 89 (2), 147-55.
 43. Firooz, A., et al., Itraconazole in the treatment of cutaneous leishmaniasis. *Int J Dermatol* **2006**, 45 (12), 1446-7.
 44. Baroni, A., et al., Cutaneous leishmaniasis treated with itraconazole. *Dermatol Ther* **2009**, 22 Suppl 1, S27-9.
 45. Saleem, K.; Rahman, A., Comparison of oral itraconazole and intramuscular meglumine antimoniate in the treatment of cutaneous leishmaniasis. *J Coll Physicians Surg Pak* **2007**, 17 (12), 713-6.
 46. Paniz Mondolfi, A. E., et al., Successful treatment of Old World cutaneous leishmaniasis caused by *Leishmania infantum* with posaconazole. *Antimicrob Agents Chemother* **2011**, 55 (4), 1774-6.
 47. Aronson, N., et al., Diagnosis and Treatment of Leishmaniasis: Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). *Am J Trop Med Hyg* **2017**, 96 (1), 24-45.
 48. Osman, O. F., et al., Use of the polymerase chain reaction to assess the success of visceral leishmaniasis treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1998**, 92 (4), 397-400.
 49. Despommier, D., et al., The role of ecotones in emerging infectious diseases. *Ecohealth* **2006**.
 50. Souza, N. A., et al., Seasonality of *Lutzomyia intermedia* and *Lutzomyia whitmani* (Diptera: Psychodidae: Phlebotominae), occurring sympatrically in area of cutaneous leishmaniasis in the State of Rio de Janeiro, Brazil. *Memorias do Instituto Oswaldo Cruz* **2002**, 97 (6), 759-65.
 51. Banks, S. D., et al., Insecticide-treated clothes for the control of vector-borne diseases: a review on effectiveness and safety. *Med Vet Entomol* **2014**, 28 Suppl 1, 14-25.
 52. Moore, S. J., et al., Insect bite prevention. *Infect Dis Clin North Am* **2012**, 26 (3), 655-73.
 53. Coler, R. N.; Reed, S. G., Second-generation vaccines against leishmaniasis. *Trends in parasitology* **2005**, 21 (5), 244-9.
 54. Kumar, R.; Engwerda, C., Vaccines to prevent leishmaniasis. *Clin Transl Immunology* **2014**, 3 (3), e13.
 55. Dinc, R., Leishmania Vaccines: the Current Situation with Its Promising Aspect for the Future. *Korean J Parasitol* **2022**, 60 (6), 379-391.(3), e13.



Amico Bignami, M.D. (1862–1929)

Bignami (\BEN-yah-meh\) collaborated with Ettore Marchiafava and Giovanni Grassi in 1892 and elucidated the main clinical features of “malignant” malaria (*Plasmodium falciparum*). Bignami also demonstrated that the “crescent” stage (gametocyte) did not cause illness. In 1898, Bignami, and Grassi worked with Antonio Dionisi and Guiseppe Bastianelli to prove that anopheline mosquitoes transmit human malaria.



Theodor Maximilian Bilharz, M.D. (1825–1862)

Bilharz described the adult stage of *Hymenolepis nana* and *Schistosoma haematobium* in human patients that had come to autopsy while he was serving in the German Army medical corps as lieutenant colonel and chief of surgery at Kasr El Aini Hospital in Cairo, Egypt. In collaboration with Karl von Siebold, he made the connection between blood in the urine and infection with *S. haematobium*.

5. Visceral Leishmaniasis

Pronunciation: \lēsh-'ma-nē-ə\

Leishmania (L.) donovani
(Ross 1903)

Leishmania (L.) infantum
(Cunha and Chagas 1937)

Introduction

Compared to the many species of *Leishmania* (LEESH-ma-NEE-ah) that cause cutaneous disease, visceral leishmaniasis (VL) is primarily caused by only two species within the *Leishmania (L.) donovani* complex: *L. (L.) donovani* and *Leishmania (L.) infantum*.¹ Previously, a third species of *Leishmania*, *Leishmania (L.) chagasi*, was identified, but this organism is now considered the same species as *L. (L.) infantum*.^{2, 3} In Europe, Africa, and Asia, *L. (L.) donovani* and *L. (L.) infantum* are found in well-defined geographical distributions. Most New World VL is caused by *L. (L.) infantum*.⁴ In this invasive form of leishmaniasis, *Leishmania* promastigotes infect macrophages, transform into amastigotes, and infect multiple cell types in which they reproduce, causing a series of

often fatal diseases, collectively referred to as VL. A few other species, such as *Leishmania (L.) tropica*, *Leishmania (L.) amazonensis*, and *Leishmania (L.) Mexicana*, can also rarely visceralize.⁵⁻⁹ The illness is characterized by hepatosplenomegaly and high fever. VL is especially prevalent in children.¹⁰ ¹¹ Estimates vary regarding the number of new cases of VL and the number of deaths due to VL.^{4, 12, 13} While cases and fatalities are felt to be underreported, it is suggested that most disease occurs in a concentrated area in Northern India, Nepal, and Bangladesh.^{13, 14}

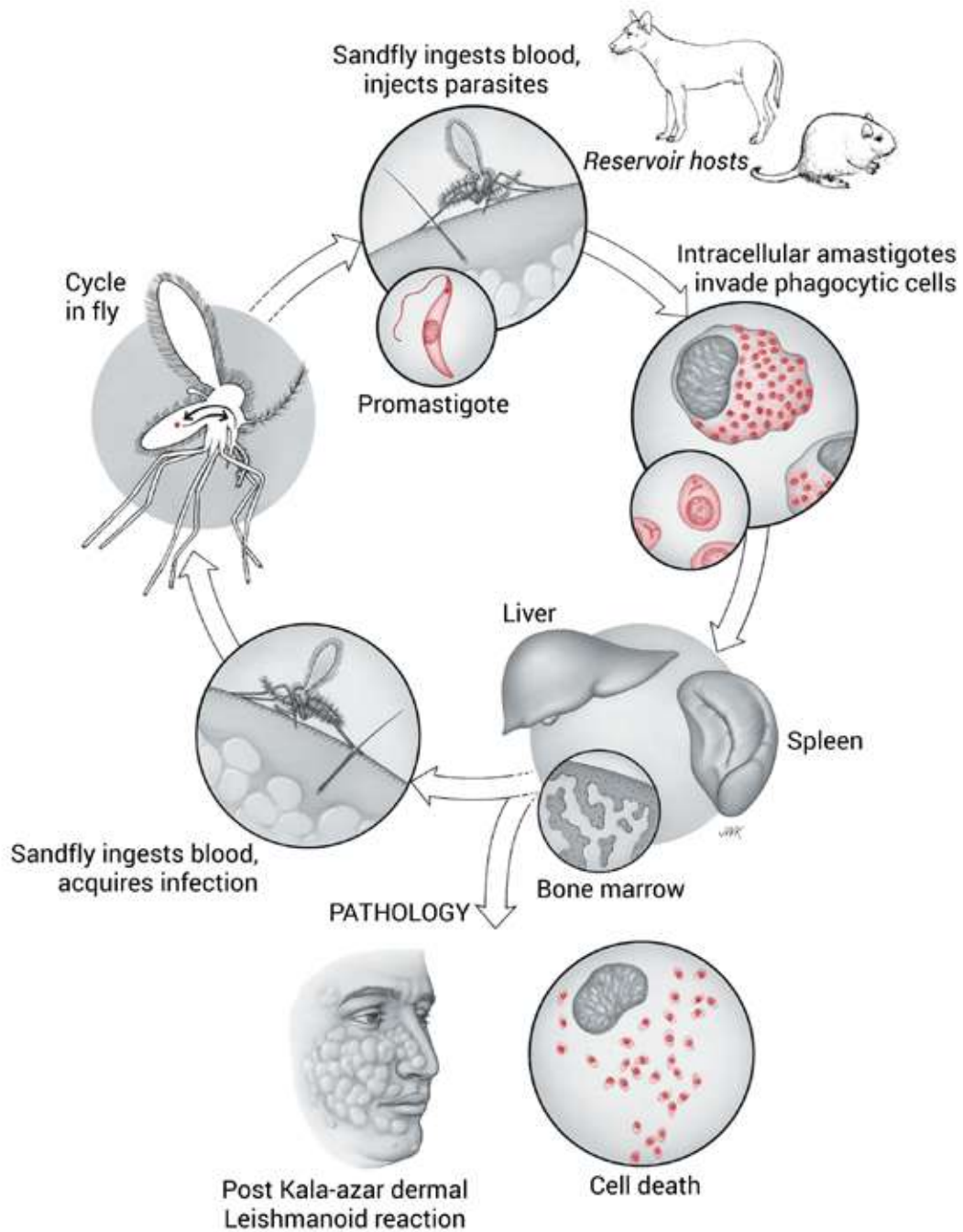
As with all other *Leishmania* species, the VL vectors are numerous species of sand flies. Humans are the primary source of *L. (L.) donovani* infection, although domestic dogs may act as reservoir hosts.¹⁵ In Northern India, Nepal, and Bangladesh, the cycle is almost entirely anthroponotic. At the same time, in other areas, such as East Africa, there are significant roles for a wide variety of reservoir hosts, including the domestic dog and numerous rodent species.

Concerning the presence of VL in the New World, an earlier hypothesis suggested that *L. (L.) infantum* had been introduced into the New World several million years ago based on it being found in a fox species native to the remote inner Amazon River basin.¹⁶ The currently accepted theory based on genetic analysis is that it was introduced during European colonization.¹⁷⁻¹⁹ The formerly named *Leishmania (L.) chagasi* and *L. (L.) infantum* are the same parasite but have some genetic differences.¹⁹ *L. (L.) infantum* is found in peri-domestic habitats, infecting domestic dogs and humans. In addition, *L. (L.) infantum* occurs in a broad range of natural environments, infecting a wide variety of wild mammals in dense forest areas, providing ample biological opportunities for eventual radiation into numerous new varieties and perhaps even new species. *L. (L.) donovani* does not appear to be closely related to any Western



Figure 5.1. Hepatosplenomegaly due to infection with *Leishmania (L.) donovani*.

Leishmania donovani



Hemisphere species of *Leishmania*, despite its minicircle DNA showing strong homology with New World species.²⁰

Historical Information

In 1903, *L. (L.) donovani* was described by two physicians, William Leishman and Charles Donovan, while they were working in separate locations in India.^{21, 22} Leishman was an officer in the British Army, and Donovan served as a physician in the Indian Medical Service. Ronald Ross, then the editor of the British Medical Journal, reviewed their separate manuscript submissions, recognized that they had observed the same disease, and named the genus and species after them in recognition of their landmark discovery. Five years later, in 1908, Charles Nicolle discovered that other mammals, particularly dogs, could also become infected.²³ In 1942, C.S. Swaminath and colleagues working in India, using human volunteers, showed that sand flies of the genus *Phlebotomus* transmitted the infection.²⁴

In 1913, Lewis E. Migone described a single case of what was most probably American VL in a patient in Paraguay. In 1937, A.M. Cunha and Carlos Chagas classified it and named it *Leishmania (L.) chagasi*.²⁵ It is now known that *Leishmania (L.) chagasi* is the same parasite as *L. (L.) infantum*.¹⁹

Life Cycle

Infection begins with the bite of an infected sand fly (Fig. 2.1). The promastigotes enter the subcutaneous tissues, attach to the extracellular matrix, and are eventually taken up by dendritic cells and macrophages (Fig. 2.3).²⁶ The parasites transform into amastigotes and divide, eventually killing the host cell. The released amastigotes circulate or are carried by infected cells to new body areas, where macrophages again become infected. The ability of some *Leishmania*

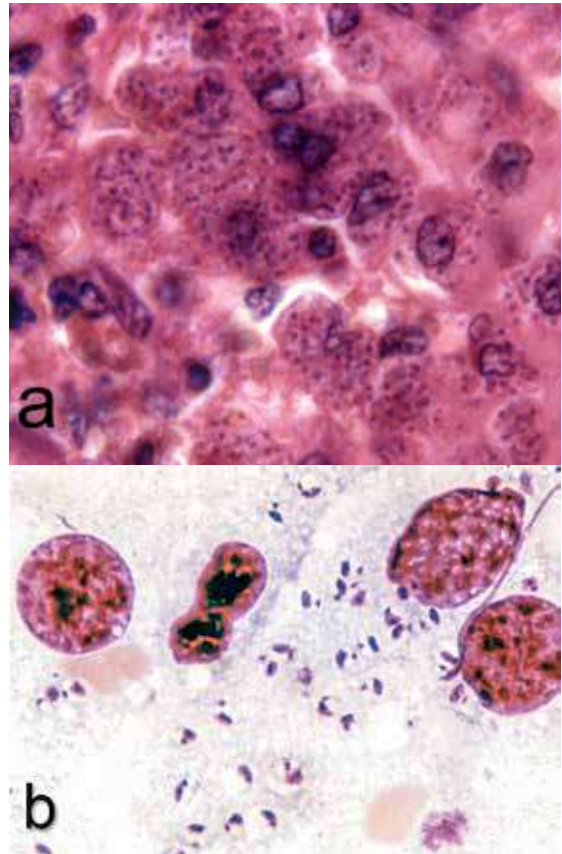


Figure 5.2a. Biopsy of liver positive for amastigotes of *L. (L.) donovani*.

Figure 5.2b. Bone marrow aspirate positive for amastigotes of *L. (L.) donovani*.

spp. to visceralize, in contrast to those species restricted to the skin, may relate to the VL type's survival at higher body temperatures.²⁷ In some mouse models, dendritic cells increase in lymphoid tissue and eventually become heavily infected but do not appear to be involved in transporting the organisms away from the bite site.²⁶

The entire reticuloendothelial system eventually becomes involved, compromising the host's ability to mount an effective immune attack against other invading microbes. The spleen, liver, and bone marrow are the most seriously affected organs (Fig. 5.1). Amastigotes have been observed in peripheral blood in greater densities at night than during daylight hours, a biological phenomenon similar to that found in periodic filariasis.²⁸ Since sand flies bite more frequently at night, this

diurnal rhythm makes acquiring parasites by the vector more likely.

The cycle is completed when an infected human is bitten by an uninfected sand fly, which ingests macrophages containing the amastigotes or free amastigotes in the blood.²⁸ The duration of the cycle within the fly is approximately ten days. Rarely, *L. (L.) donovani* has been transmitted by organ transplantation.²⁹

Immunity depends upon Th1 responses, and loss of or decreased Th1 response to *Leishmania* spp. may be critical to the development of VL.³⁰ T cells producing interferon- γ , TNF- α , and interleukin-2 activated macrophages kill intracellular parasites by NO synthesis.³¹ Despite high levels of IgG and IgE being generated during infection, there is no evidence that these are functional or playing any significant role in protection.³²

Clinical Disease

Kala-azar (Old World Visceral Leishmaniasis)

Although not directly translatable from modern standardized Hindi, the term “Kala-azar” appears to have derived originally from the Hindustani (Assamese) words ‘kala’ referring to black, and ‘azar’ referring to fever or sickness.³³ Kala-azar is roughly translated as “black fever” because of the appearance of the febrile patient’s skin after an infection. Most cases of Kala-azar have an incubation period of 3–6 months.³³ A large number of infected patients remain asymptomatic with variable frequency that may be due to parasite virulence, host genetics, nutritional status, and age of the infected host.³⁴⁻³⁷ A study of asymptomatic troops returning from Iraq documented evidence of visceral leishmaniasis in nearly 20% and highlights the need for long-term follow-up to determine risk factors for reactivation and

appropriate treatment.³⁸ When the disease does manifest, the onset can be gradual or sudden.³³ In the former, the initial symptoms can be nonspecific, often relating to splenomegaly, which may eventually occupy most of the left side of the abdomen. The clinical diagnosis is often missed or is discovered by accident when patients present with other acute illnesses, such as a respiratory or gastrointestinal infection.³⁹

The onset of the disease is accompanied by high fever, which may be irregular, often giving rise to a characteristic double daily spike. Fever is intermittent, subsiding for days or weeks only to return, and resembles the pattern seen in patients suffering from undulant fever. Though weak, the infected individual does not necessarily feel ill and tolerates bouts of fever, distinguishing them from those with typhoid fever, tuberculosis, or malaria. Diagnosing the disease can require a high index of suspicion, as many presentations are similar to the other febrile disorders common in the areas where VL is endemic.⁴⁰ Generalized lymphadenopathy, and later hepatosplenomegaly, eventually develop. The classic darkening of the skin associated with this disease is seen in different areas where VL occurs at variable frequencies.⁴¹

VL is progressive, and the individual eventually suffers the consequences of a compromised immune system. Many people die with Kala-azar rather than from it because of the acquisition of intercurrent infections. HIV-1 infection notably accelerates the overall immune dysfunction when co-infection is present.⁴²

As VL progresses, patients may develop anemia, low platelet levels (thrombocytopenia), hepatosplenomegaly, and a drop in hemoglobin.⁴⁰ High serum protein concentrations, approaching ten g/dl, are due almost

exclusively to a rise in non-protective IgG resulting from a polyclonal activation of B cells.^{32, 43} Patients infected with HIV/AIDS and VL due to *L. (L.) donovani* and *L. (L.) infantum* sometimes present with cutaneous lesions in which organisms can be demonstrated, in addition to the involvement of the reticuloendothelial system.^{44, 45}

Congenital Kala-azar

Infants born to mothers with Kala-azar can acquire the infection *in utero*.⁴⁶ Organisms are brought to the developing fetus by infected macrophages. The pathologies and clinical presentations of congenital VL in newborns are similar to those of the adult form.⁴⁷

Post-Kala-azar Dermal Leishmaniasis

Some treated patients develop a skin rash six months to several years after the treatment of VL.¹² A hypo-pigmented or erythematous macular rash that eventually becomes papular and nodular is characteristic of this condition, known as post-Kala-azar dermal leishmaniasis (PKDL).⁴⁸ The rash and associated lesions are particularly prominent on the face and the upper parts of the body, resembling lesions of lepromatous leprosy. The lesions are filled with histiocytes containing amastigotes and serve as sources of infection for sand flies. There may be neurological damage.⁴⁹ Among African patients, only 2% of those who recover develop such skin lesions. Involvement of the eye is also common.⁵⁰ PKDL is strongly associated with an interleukin-10-driven (Th2) cytokine pattern.⁵¹⁻⁵³ Individuals with PKDL are a concern in areas where anthroponotic transmission is significant, as these individuals are thought to be highly efficient disease spreaders.¹²

Atypical CL has also been described for infections caused by *L. (L.) infantum*; it may either be a form of PKDL following a sub-clinical infection or a form of Kala-azar that

presents solely with cutaneous manifestations.⁵⁴

Diagnosis (see Clinical Appendix)

Definitive diagnosis of VL depends upon the isolation of the organism or NAATs.⁵⁵⁻⁵⁷ PKDL can be diagnosed by NAAT with high sensitivity.⁵⁸ Specimens for testing can be obtained from either bone marrow or splenic aspiration. The higher-risk procedure of splenic aspiration is used less often since similar results can be achieved with large-volume bone marrow aspirations and NAAT testing. Samples of bone marrow or splenic aspiration can be sent for culture, microscopic evaluation, and NAAT.⁵⁹⁻⁶² Culture of bone marrow aspirates effectively reveals the organisms, but it takes three to four days for the parasites to grow significantly. Parasites can also be seen on microscopy of sectioned or biopsied material (Figs. 5.2a, 5.2b). Additional tests, such as serum antibody, urine antigen, and the leishmanin test, are no longer routinely used. In their place, the highly sensitive and highly specific rK39 antigen ELISA is now widely available and used in many settings.^{63, 64} Also, a simple point-of-care lateral flow rK39 assay is currently utilized in many endemic areas.⁶⁵

Treatment (see Clinical Appendix)

Unlike cutaneous leishmaniasis, identification of the specific species is not critical. The treatment of choice for VL is systemic therapy. Liposomal amphotericin B is currently the drug of choice for VL in India due to its safety profile, high efficacy, and the development of resistance to other therapies.⁶⁶ The liposomal form of amphotericin is better tolerated, has a better safety profile, and has improved bioavailability compared to amphotericin. It is taken up by the phagocytic cells infected by the *Leishmania* amas-

tigotes.⁶⁷ Other amphotericin B formulations are also in development.⁶⁸

Sodium stibogluconate had been preferred in East Africa for Kala-azar patients, and now combination therapy with paromomycin is recommended. However, it requires 17 days of painful injections.⁶⁹⁻⁷² Continued usage of antimonials depends on regional resistance rates (high in India) and access to alternative therapies.⁶⁶ Miltefosine is an oral agent available as a secondary choice for systemic treatment of VL, and further studies will help define its role in the treatment of childhood VL.^{73, 74} Miltefosine is an oral agent available as an option for systemic therapy of VL with increasing data describing use in HIV patients⁷⁶, in refractory disease when used with paromomycin, and a study establishing a dose for pediatric use in East Africa.⁷⁷ However, Phase 2 combination studies in East Africa demonstrated lower cure rates with several combinations, such as miltefosine and liposomal amphotericin B, than was expected based on the experience in India.⁷⁹ Pentamidine and sitamaquine (another oral agent) are alternatives that may end up having a role in the therapy of VL.⁸⁰ Response to any treatment is generally assessed clinically with resolution of fever, reduced splenic size, and weight gain. The HIV status of the patient impacts recommendations, and secondary prophylaxis, along with adherence to highly active antiretroviral therapy, is recommended for patients with CD4 counts < 200 cells per microL. There is a high rate of relapse in HIV/VL coinfecting individuals.⁷⁵ With advances in bioinformatics and machine learning, new drugs for VL could rapidly accelerate.⁸⁰ Consulting tropical diseases working group guidelines is essential when considering the choice of medicines, managing frequent adverse drug reactions, and optimizing care in various adult and pediatric patient populations.⁸¹

Prevention and Control

Infection due to *L. (L.) donovani* and *L. (L.) infantum* differ regarding the source or reservoir of infection. Transmission of *L. (L.) donovani* is largely anthroponotic, while mammals such as the domestic dog are major reservoirs of infection for *L. infantum*. There is limited evidence to date for the effective implementation of sand fly or reservoir control programs, emphasizing the neglected nature of this disease. India has had limited success in controlling VL despite progress against other neglected diseases, such as Yaws.^{82, 83}

Political unrest, forced migration, and prolonged periods of drought have resulted in several situations where large numbers of patients have become afflicted with Kala-azar. These situations concentrate people in areas that favor high transmission rates. The unavailability of insecticides, insect repellents, and bed netting, as well as the general lack of proper attention to health care needs during times of high stress, often allow vector-borne diseases to “have their way” with refugees, and *Leishmania* spp. are no exception.

Attempts to control *L. (L.) infantum* transmission by controlling its spread in domestic dogs throughout the Mediterranean basin has met with a singular lack of success, and large-scale culling of dogs, done in the past, is felt by many to be unacceptable.¹⁵ Leishmaniasis due to *L. (L.) infantum* in domestic and wild dogs has been described in the United States.⁸⁴ No human cases have been reported from this area of the world yet. The proper vector species of sandflies are present, and it is probably just a matter of time before a human outbreak occurs.

There is continued work on vaccines. First-

generation vaccines using whole-killed or purified fractions, genetically modified attenuated vaccines, DNA vaccines, and sand fly saliva vaccines are all being explored.^{85, 86} As new-generation adjuvant candidates become available, there is optimism that previous vaccine targets for VL could be improved.⁸⁷ Additionally, adding biologics to current chemotherapy regimens could improve therapeutic outcomes.⁸⁸ A canine vaccine exists, and there are recommendations regarding

protecting dogs from infection and improving their health and nutrition.⁸⁹ Remote sensing efforts have identified rainfall and altitude as the two most critical environmental variables in predicting outbreaks of VL in certain parts of the world.⁹⁰ It is hoped that future studies using this powerful new technology will result in an even greater application of satellite data to augment leishmaniasis control in other parts of the world.

References

1. Chappuis, F., et al., Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? *Nat Rev Microbiol* **2007**, *5* (11), 873-82.
2. Dantas-Torres, F., *Leishmania infantum* versus *Leishmania chagasi*: do not forget the law of priority. *Mem Inst Oswaldo Cruz* **2006**, *101* (1), 117-8; discussion 118.
3. Dantas-Torres, F., Final comments on an interesting taxonomic dilemma: *Leishmania infantum* versus *Leishmania infantum chagasi*. *Mem Inst Oswaldo Cruz* **2006**, *101* (8), 929-30.
4. Ready, P. D., Epidemiology of visceral leishmaniasis. *Clin Epidemiol* **2014**, *6*, 147-54.
5. Berman, J., Recent Developments in Leishmaniasis: Epidemiology, Diagnosis, and Treatment. *Current infectious disease reports* **2005**, *7* (1), 33-38.
6. Pastor-Santiago, J. A., et al., American visceral leishmaniasis in Chiapas, Mexico. *Am J Trop Med Hyg* **2012**, *86* (1), 108-14.
7. Monroy-Ostria, A., et al., Aetiology of visceral leishmaniasis in Mexico. *Acta Trop* **2000**, *75* (2), 155-61.
8. Magill, A. J., et al., Visceral infection caused by *Leishmania tropica* in veterans of Operation Desert Storm. *N Engl J Med* **1993**, *328* (19), 1383-7.
9. Magill, A. J., et al., Visceral infection caused by *Leishmania tropica* in veterans of Operation Desert Storm. *The New England journal of medicine* **1993**, *328* (19), 1383-7.
10. Maltezou, H. C., et al., Visceral leishmaniasis during childhood in southern Greece. *Clin Inf Dis* **2000**, *31* (5), 1139-43.
11. Haidar, N. A., et al., Visceral Leishmaniasis in children in the Yemen. *Saudi medical journal* **2001**, *22* (6), 516-9.
12. Desjeux, P., et al., Report of the Post Kala-azar Dermal Leishmaniasis (PKDL) Consortium Meeting, New Delhi, India, 27-29 June 2012. *Parasit Vectors* **2013**, *6*, 196.
13. Scarpini, S., et al., Visceral Leishmaniasis: Epidemiology, Diagnosis, and Treatment Regimens in Different Geographical Areas with a Focus on Pediatrics. *Microorganisms* **2022**, *10* (10).
14. Alvar, J., et al., Leishmaniasis worldwide and global estimates of its incidence. *PLoS One* **2012**, *7* (5), e35671.
15. Dantas-Torres, F., et al., Canine Leishmaniasis Control in the Context of One Health. *Emerg Infect Dis* **2019**, *25* (12), 1-4.
16. Lainson, R., et al., New World Leishmaniasis-The Neotropical *Leishmania* Species. 2005.
17. Mauricio, I. L., et al., Genomic diversity in the *Leishmania donovani* complex. *Parasitology* **1999**, *119* (Pt 3), 237-46.
18. I., J. M. A. M.; Mauricio, I. L., The strange case of *Leishmania chagasi*. *Parasitol Today* **2000**, *16*, 188-189.
19. de Freitas Milagres, T., et al., Same parasite, different outcomes: unraveling the epidemiology of *Leishmania infantum* infection in Brazil and Spain. *Trends Parasitol* **2023**, *39* (9), 774-785.
20. Singh, N., et al., Characterization of kinetoplast DNA minicircles of an Indian isolate of

- Leishmania donovani*. *Acta tropica* **1999**, 73 (3), 313-9.
21. Leishman, W. B., On the possibility of occurrence of trypanosomiasis in India. *Bmj* **1903**, 1, 1252-1254.
 22. Donovan, C., On the possibility of occurrence of trypanosomiasis in India. *BMJ* **1903**, 2
 23. Nicolle, C., Nouvelles acquisitions sur kala-azar: cultures: inoculation au chien: etiologie. . *Hebdom Sci* **1908**, 146, 498-499.
 24. Swaminath, C. S., et al., Transmission of Indian kala-azar to man by the bites of *Phlebotomus argentipes*. *Ann Brun Indian Res* **1942**, 30, 473-477.
 25. Cunha, A. M.; Chagas, E., Nova especie de protozoario do genero *Leishmania* patogenico para o homen. *Leishmania chagasi n spp Nota PreviaHospital Rio de Janerio* **1937**, 11, 3-9.
 26. Baldwin, T., et al., Dendritic cell populations in *Leishmania major*-infected skin and draining lymph nodes. *Infection and immunity* **2004**, 72 (4), 1991-2001.
 27. Callahan, H. L., et al., *Leishmania* spp: temperature sensitivity of promastigotes in vitro as a model for tropism in vivo. *Experimental parasitology* **1996**, 84 (3), 400-9.
 28. Saran, R., et al., Diurnal periodicity of *Leishmania* amastigotes in peripheral blood of Indian Kala-azar patients. *Acta tropica* **1997**, 68 (3), 357-60.
 29. Hernandez-Perez, J.; Yebra-Bango, M., Visceral Leishmaniasis (kala-azar) in solid organ transplantation: report of five cases and review. *Clin Infect Dis* **1999**, 29, 918-21.
 30. Bern, C., et al., Loss of leishmanin skin test antigen sensitivity and potency in a longitudinal study of visceral leishmaniasis in Bangladesh. *Am J Trop Med Hyg* **2006**, 75 (4), 744-8.
 31. Arora, S. K., et al., *Leishmania donovani*: identification of novel vaccine candidates using human reactive sera and cell lines. *Experimental parasitology* **2005**, 109 (3), 163-70.
 32. Ravindran, R., et al., Characterization of immunoglobulin G and its subclass response to Indian kala-azar infection before and after chemotherapy. *Infection and immunity* **2004**, 72 (2), 863-70.
 33. Herwaldt, B. L., Leishmaniasis. *Lancet (London, England)* **1999**, 354 (9185), 1191-9.
 34. Singh, O. P., et al., Asymptomatic *Leishmania* infection: a new challenge for *Leishmania* control. *Clin Infect Dis* **2014**, 58 (10), 1424-9.
 35. Das, S., et al., Asymptomatic *Leishmania* infections in northern India: a threat for the elimination programme? *Trans R Soc Trop Med Hyg* **2014**, 108 (11), 679-84.
 36. Clemente, W. T., et al., High prevalence of asymptomatic *Leishmania* spp. infection among liver transplant recipients and donors from an endemic area of Brazil. *Am J Transplant* **2014**, 14 (1), 96-101.
 37. Picado, A., et al., Risk factors for visceral leishmaniasis and asymptomatic *Leishmania donovani* infection in India and Nepal. *PloS one* **2014**, 9 (1), e87641.
 38. Mody, R. M., et al., Asymptomatic Visceral *Leishmania infantum* Infection in US Soldiers Deployed to Iraq. *Clin Infect Dis* **2019**, 68 (12), 2036-2044.
 39. Badaro, R., et al., New perspectives on a subclinical form of visceral leishmaniasis. *The Journal of infectious diseases* **1986**, 154 (6), 1003-11.
 40. Tanoli, Z. M., et al., Clinical presentation and management of visceral leishmaniasis. *J Ayub Med Coll Abbottabad* **2005**, 17 (4), 51-3.
 41. Seaman, J., et al., Epidemic visceral leishmaniasis in southern Sudan: treatment of severely debilitated patients under wartime conditions and with limited resources. *Ann Intern Med* **1996**, 124 (7), 664-72.
 42. Ritmeijer, K., et al., Ethiopian visceral leishmaniasis: generic and proprietary sodium stibogluconate are equivalent; HIV co-infected patients have a poor outcome. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2001**, 95 (6), 668-72.
 43. Galvao-Castro, B., et al., Polyclonal B cell activation, circulating immune complexes and autoimmunity in human american visceral leishmaniasis. *Clin Exp Immunol* **1984**, 56 (1), 58-66.
 44. Alvar, J., et al., *Leishmania* and human immunodeficiency virus coinfection: the first 10 years. *Clinical microbiology reviews* **1997**, 10 (2), 298-319.
 45. Agostoni, C., et al., Mediterranean leishmaniasis in HIV-infected patients: epidemiological, clinical, and diagnostic features of 22 cases. *Infection* **1998**, 26 (2), 93-9.

46. Eltoun, I. A., et al., Congenital kala-azar and leishmaniasis in the placenta. *The American journal of tropical medicine and hygiene* **1992**, *46* (1), 57-62.
47. Meinecke, C. K., et al., Congenital transmission of visceral leishmaniasis (Kala Azar) from an asymptomatic mother to her child. *Pediatrics* **1999**, *104* (5), e65.
48. Beena, K. R., et al., Identification of parasite antigen, correlation of parasite density and inflammation in skin lesions of post kala-azar dermal leishmaniasis. *Journal of cutaneous pathology* **2003**, *30* (10), 616-20.
49. Khandpur, S., et al., Nerve involvement in Indian post kala-azar dermal leishmaniasis. *Acta dermato-venereologica* **2004**, *84* (3), 245-6.
50. el Hassan, A. M., et al., Post kala-azar ocular leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1998**, *92* (2), 177-9.
51. Gasim, S., et al., High levels of plasma IL-10 and expression of IL-10 by keratinocytes during visceral leishmaniasis predict subsequent development of post-kala-azar dermal leishmaniasis. *Clinical and experimental immunology* **1998**, *111* (1), 64-9.
52. Kharazmi, A., et al., T-cell response in human leishmaniasis. *Immunology letters* **1999**, *65* (1-2), 105-8.
53. Atta, A. M., et al., Anti-leishmanial IgE antibodies: a marker of active disease in visceral leishmaniasis. *The American journal of tropical medicine and hygiene* **1998**, *59* (3), 426-30.
54. Ponec, J., et al., Failure to demonstrate natriuretic activity in the posterior pituitary after immunoneutralization of the vasopressin content. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme* **1991**, *23* (10), 473-5.
55. Andresen, K., et al., Diagnosis of visceral leishmaniasis by the polymerase chain reaction using blood, bone marrow and lymph node samples from patients from the Sudan. *Tropical medicine & international health : TM & IH* **1997**, *2* (5), 440-4.
56. Minodier, P., et al., Pediatric visceral leishmaniasis in southern France. *The Pediatric infectious disease journal* **1998**, *17* (8), 701-4.
57. Sinha, P. K., et al., Diagnosis & management of leishmania/HIV co-infection. *The Indian journal of medical research* **2005**, *121* (4), 407-14.
58. Osman, O. F., et al., Use of PCR for diagnosis of post-kala-azar dermal leishmaniasis. *Journal of clinical microbiology* **1998**, *36* (6), 1621-4.
59. da Silva, M. R., et al., Sensitivity of bone marrow aspirates in the diagnosis of visceral leishmaniasis. *Am J Trop Med Hyg* **2005**, *72* (6), 811-4.
60. Lightner, L. K., et al., Comparison of microscopy and culture in the detection of *Leishmania donovani* from splenic aspirates. *Am J Trop Med Hyg* **1983**, *32* (2), 296-9.
61. Chulay, J. D.; Bryceson, A. D., Quantitation of amastigotes of *Leishmania donovani* in smears of splenic aspirates from patients with visceral leishmaniasis. *Am J Trop Med Hyg* **1983**, *32* (3), 475-9.
62. Mbui, J., et al., A simple and inexpensive medium for culture of splenic aspirates in visceral leishmaniasis. *East Afr Med J* **1999**, *76* (6), 358.
63. Quinnell, R. J., et al., Evaluation of rK39 rapid diagnostic tests for canine visceral leishmaniasis: longitudinal study and meta-analysis. *PLoS Negl Trop Dis* **2013**, *7* (1), e1992.
64. Cunningham, J., et al., A global comparative evaluation of commercial immunochromatographic rapid diagnostic tests for visceral leishmaniasis. *Clin Infect Dis* **2012**, *55* (10), 1312-9.
65. Boelaert, M., et al., Rapid tests for the diagnosis of visceral leishmaniasis in patients with suspected disease. *Cochrane Database Syst Rev* **2014**, *6*, CD009135.
66. Freitas-Junior, L. H., et al., Visceral leishmaniasis treatment: What do we have, what do we need and how to deliver it? *Int J Parasitol Drugs Drug Resist* **2012**, *2*, 11-9.
67. Torchilin, V. P., Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov* **2005**, *4* (2), 145-60.
68. Kumar, P., et al., Limitations of current chemotherapy and future of nanoformulation-based AmB delivery for visceral leishmaniasis-An updated review. *Front Bioeng Biotechnol* **2022**, *10*, 1016925.
69. Karki, P., et al., A thirty day course of sodium stibogluconate for treatment of Kala-azar in Nepal.

- The Southeast Asian journal of tropical medicine and public health* **1998**, 29 (1), 154-8.
70. Lira, R., et al., Evidence that the high incidence of treatment failures in Indian kala-azar is due to the emergence of antimony-resistant strains of *Leishmania donovani*. *The Journal of infectious diseases* **1999**, 180 (2), 564-7.
 71. Kimutai, R., et al., Safety and Effectiveness of Sodium Stibogluconate and Paromomycin Combination for the Treatment of Visceral Leishmaniasis in Eastern Africa: Results from a Pharmacovigilance Programme. *Clin Drug Investig* **2017**, 37 (3), 259-272.
 72. Alves, F., et al., Recent Development of Visceral Leishmaniasis Treatments: Successes, Pitfalls, and Perspectives. *Clin Microbiol Rev* **2018**, 31 (4).
 73. World Health, O., Control of the leishmaniasis. *World Health Organ Tech Rep Ser* **2010**, (949), xii-xiii, 1-186, back cover.
 74. Bhattacharya, S. K., et al., Efficacy and tolerability of miltefosine for childhood visceral leishmaniasis in India. *Clin Inf Dis* **2004**, 38 (2), 217-21.
 75. Costa, L., et al., Factors associated with relapse and hospital death in patients coinfecting with visceral leishmaniasis and HIV: a longitudinal study. *BMC Infect Dis* **2023**, 23 (1), 141.
 76. Burki, T., Guidelines for visceral leishmaniasis and HIV co-infection. *Lancet Infect Dis* **2022**, 22 (8), 1124-1125.
 77. Musa, A. M., et al., Paromomycin and Miltefosine Combination as an Alternative to Treat Patients With Visceral Leishmaniasis in Eastern Africa: A Randomized, Controlled, Multicountry Trial. *Clin Infect Dis* **2023**, 76 (3), e1177-e1185.
 78. Mbui, J., et al., Pharmacokinetics, Safety, and Efficacy of an Allometric Miltefosine Regimen for the Treatment of Visceral Leishmaniasis in Eastern African Children: An Open-label, Phase II Clinical Trial. *Clin Infect Dis* **2019**, 68 (9), 1530-1538.
 79. Wasunna, M., et al., Efficacy and Safety of AmBisome in Combination with Sodium Stibogluconate or Miltefosine and Miltefosine Monotherapy for African Visceral Leishmaniasis: Phase II Randomized Trial. *PLoS Negl Trop Dis* **2016**, 10 (9), e0004880.
 80. Singh, R., et al., Recent Advances in Chemotherapeutics for Leishmaniasis: Importance of the Cellular Biochemistry of the Parasite and Its Molecular Interaction with the Host. *Pathogens* **2023**, 12 (5).
 81. Aronson, N., et al., Diagnosis and Treatment of Leishmaniasis: Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). *Am J Trop Med Hyg* **2017**, 96 (1), 24-45.
 82. Gonzalez, U., et al., Vector and reservoir control for preventing leishmaniasis. *Cochrane Database Syst Rev* **2015**, 8, CD008736.
 83. Gurunath, U., et al., An overview of visceral leishmaniasis elimination program in India: a picture imperfect. *Expert Rev Anti Infect Ther* **2014**, 12 (8), 929-35.
 84. Alvar, J., et al., Canine leishmaniasis. *Advances in parasitology* **2004**, 57, 1-88.
 85. Dinc, R., Leishmania Vaccines: the Current Situation with Its Promising Aspect for the Future. *Korean J Parasitol* **2022**, 60 (6), 379-391.
 86. Coler, R. N.; Reed, S. G., Second-generation vaccines against leishmaniasis. *Trends in parasitology* **2005**, 21 (5), 244-9.
 87. Ratnapriya, S., et al., Visceral leishmaniasis: An overview of vaccine adjuvants and their applications. *Vaccine* **2019**, 37 (27), 3505-3519.
 88. Mota, C. A., et al., Three decades of clinical trials on immunotherapy for human leishmaniasis: a systematic review and meta-analysis. *Immunotherapy* **2021**, 13 (8), 693-721.
 89. Jain, K.; Jain, N. K., Vaccines for visceral leishmaniasis: A review. *J Immunol Methods* **2015**, 422, 1-12.
 90. Elnaïem, D.-E. A., et al., Risk mapping of visceral leishmaniasis: the role of local variation in rainfall and altitude on the presence and incidence of kala-azar in eastern Sudan. *The American journal of tropical medicine and hygiene* **2003**, 68 (1), 10-7.



Ann Bishop, D. Sc. (1899-1990)

Dr. Bishop began her work with ciliate parasites and worked on therapeutics for amoebic diseases; she went on to discover several new parasitic species. Bishop is most famous for her work on *Plasmodium* and early studies on drug resistance in this group of parasites. She was also committed to helping other women pursue their educational ambitions and in 1945 helped to organize the Girton College Working Women's Summer School.

Dr. Bishop not only contributed to the science of parasitology but is also credited as one of the individuals largely responsible for the creation of the British Society of Parasitology. Her commitment to the advancement of young parasitologists led to the creation of the Ann Bishop Traveling Award supporting young parasitologists traveling for fieldwork.

6. African Trypanosomiasis

Pronunciation: \tri-pan-ə- 'sō-mə\

Trypanosoma brucei gambiense

(Dutton 1902)

Pronunciation: \brū'sē-ī\gam-bē-en'sē\

Trypanosoma brucei rhodesiense

(Stephens and Fantham 1910)

Pronunciation: \brū'sē-ī\rō-dē-zē-en'sē\

Introduction

Human African sleeping sickness or human African trypanosomiasis (HAT) consists of two similar but distinct diseases. The disease is caused by two vector-borne flagellated protozoans that are able to live in the blood of mammals. *Trypanosoma brucei gambiense* (tri-PAN-oh-so-mah\BROOS-ee-eye\gam-BEE-en-see\) is the causative agent of West African trypanosomiasis) and *T. b. rhodesiense* (row-DEE-zee-en-see\) causes East African trypanosomiasis. The bite of the tsetse fly spreads both types of HAT. West African and East African sleeping sickness are restricted to Africa. Some 60 million people are at risk for HAT based on the range of the tsetse fly vectors.¹ There have been several significant outbreaks, mainly due to extensive forced migrations caused by civil turmoil, which has led to the breakdown of control measures against the vector. The total number of cases each year does seem to be decreasing with the implementation of effective vector control programs.² The number of reported cases of HAT is now less than 1,000 per year, with over 95% being due to West African trypanosomiasis and about half reported from the DRC.³ *T. brucei* and related species are part of a larger group of organisms characterized by the presence of a kinetoplast (a primitive mitochondrion). They are members of the kinetoplastidae (e.g., *Leishmania* spp., *Trypanosoma cruzi*).

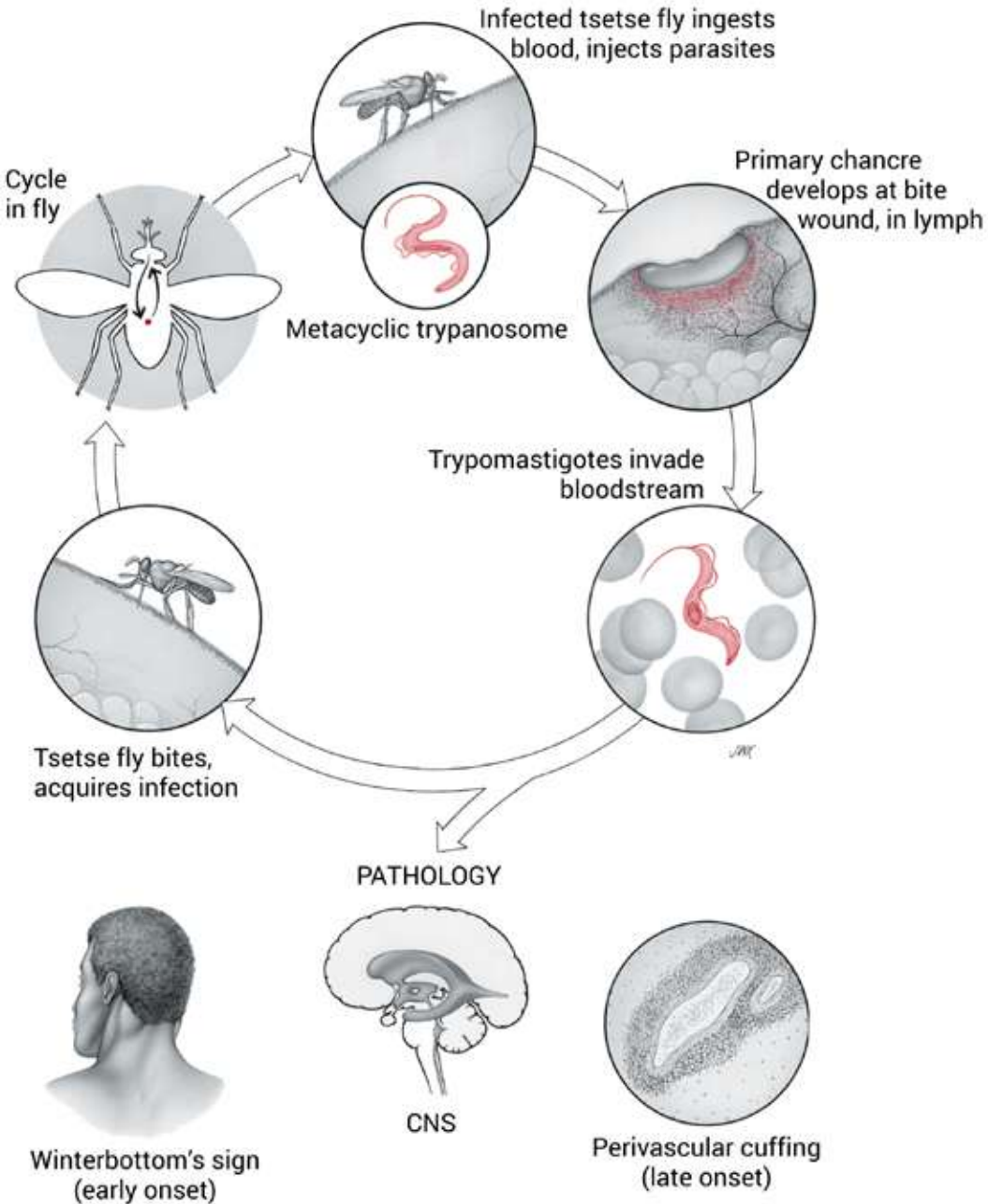
Tsetse flies of the genus *Glossina* transmit trypanosomes throughout a broad region of equatorial Africa. These are ecologically restricted to the boundaries of the Sahara Desert to the north and the dryer temperate regions south of the equator. *T. b. gambiense* is found mainly in the western and central African countries of Cameroon, Benin, Central African Republic, Gabon, Ghana, Guinea, Ivory Coast, Liberia, Nigeria, Senegal, The Gambia, Uganda, and the Democratic Republic of Congo. *T. b. rhodesiense* occurs mainly in Burundi, Botswana, Congo, Ethiopia, Kenya, Mozambique, Rwanda, Sudan, Tanzania, Uganda, Zambia, and Zimbabwe. In general, *T. b. gambiense* and *T. b. rhodesiense* are found in different geographical areas except Uganda, where both forms are present.²

The domestic pig is considered the most important reservoir host for *T. b. gambiense* in West Africa.⁴ In contrast, many species of wild animals and domestic cattle in East Africa are reservoirs for *T. b. rhodesiense*. Cases of East African sleeping sickness have occurred in travelers who entered game parks in these areas to view large wild animals.^{5,6} Trypanosomiasis can also be a prob-

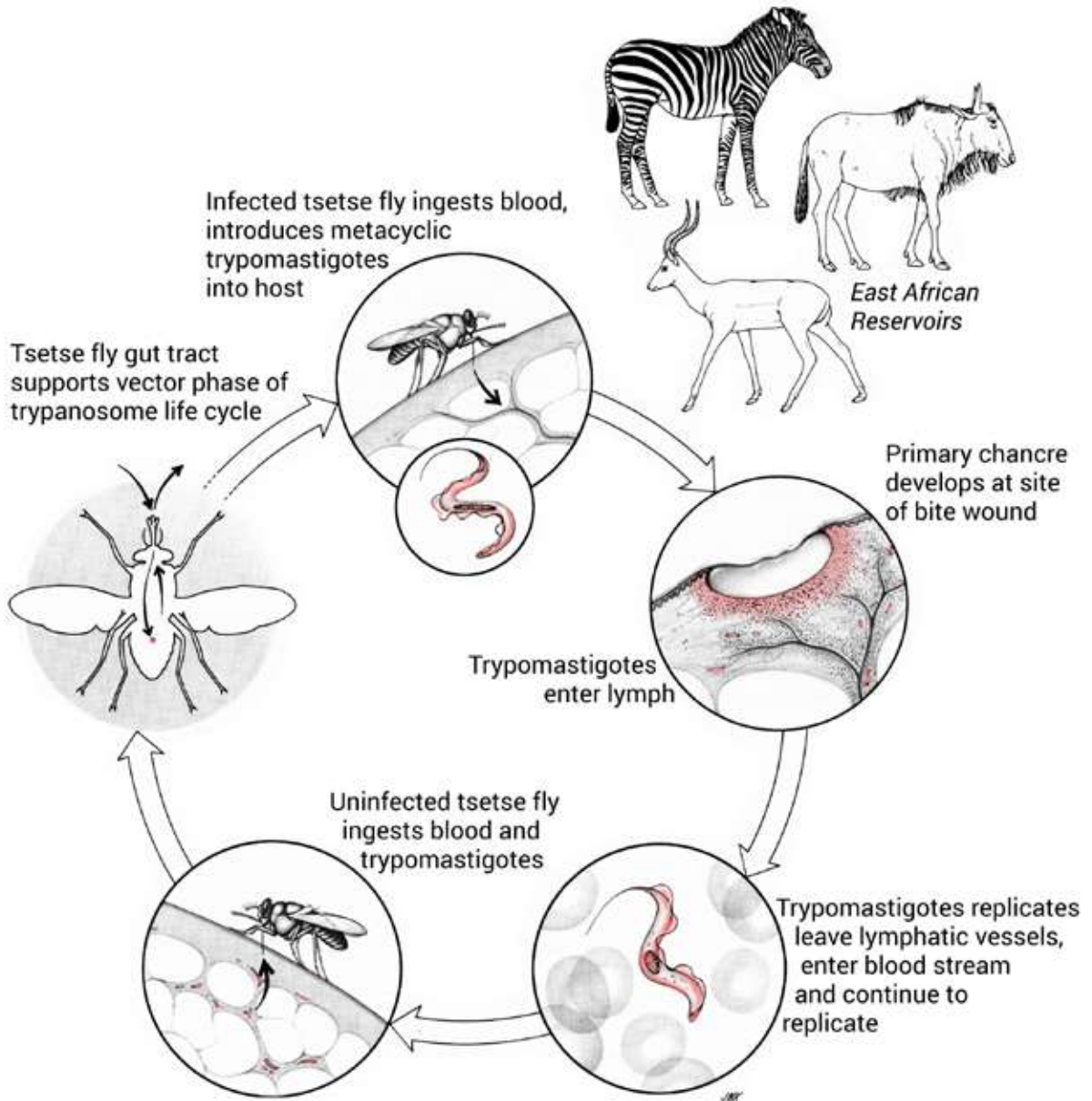


Figure 6.1. Metacyclic trypanomastigote from the tsetse fly. 20 μ m. Courtesy I. Cunningham.

Trypanosoma brucei gambiense



Trypanosoma brucei rhodesiense



lem in domestic animals imported to Africa from Europe. In addition, many species of trypanosomes related to those infecting humans cause severe disease in cattle.^{7,8} The genomes of several trypanosomes have now been sequenced.⁹⁻¹¹

Historical Information

In the 1700s, John Atkins published his observations of sleeping sickness.¹² A century later, in 1895, David Bruce described the disease and its causative agent by showing that nagana, a disease of cattle, was caused by trypanosomes and that tsetse flies were the vectors.¹³ A few years later, in 1902, Robert Forde, working in West Africa, described a clinical condition in humans similar to that in cattle caused by *T. b. gambiense*.¹⁴ In 1910, John Stephens and Harold Fantham isolated and described *T. b. rhodesiense* from human cases in East Africa.¹⁵ The two organisms, *T. b. gambiense* and *T. b. rhodesiense*, are morphologically identical. Soon after, in 1912, Allan Kinghorn and Warrington Yorke demonstrated that *T. b. rhodesiense* could be transmitted from humans to animals by tsetse flies.¹⁶ They also concluded that game animals, such as waterbuck, hartebeest, impala, and warthog, could serve as reservoir hosts for the East African trypanosome.

Dr. Thomas Masterman Winterbottom was an English physician and abolitionist who traveled to the colony of the Sierra Leone Company and spent four years in Africa. Upon



Figure 6.2. Tsetse fly taking a blood meal.



Figure 6.3. Bloodstream trypanomastigote. 15 μm .

returning to England to take over his father's medical practice, Dr. Winterbottom described diseases he had seen, including West African trypanosomiasis. He noted that the slave traders would avoid purchasing enslaved people with the characteristic posterior cervical adenopathy that is known today as Winterbottom's sign.¹⁷

Life Cycle

The biological characteristics of the two subspecies are very similar. African trypanosomes live extracellularly, both in the mammalian and insect host.¹⁸ The bloodstream form measures 15–40 μm in length (Fig. 6.1). The human host acquires the organism when the metacyclic stage is injected intradermally by the bite of an infected tsetse fly (Fig. 6.2). The organisms immediately transform into bloodstream-form trypomastigotes (long, slender forms, Fig. 6.3), and divide by binary fission in the interstitial spaces at the site of the bite.

As the result of repeated replication cycles, the buildup of metabolic wastes and cell debris occurs, leading to extensive necrosis and the formation of a soft, painless chancre at the site of the tsetse fly bite facilitated by a host T-cell response.¹⁹ Replication continues

in the blood, producing millions of trypomastigotes. During this time, the trypomastigotes behave like anaerobes, processing glucose. Trypanosomes have several intracellular inclusions: the kinetoplast-mitochondrion, the glycosome, and a multi-protein aggregate termed the editosome.²⁰⁻²⁴ One of its unusual features is that all the DNA of the mitochondrion, which can be up to 25% of the total cellular DNA, is localized in the kinetoplast adjacent to the flagellar pocket. Kinetoplast DNA exists in two forms: mini circles and maxi circles. Mini circle DNA encodes guide RNAs that direct extensive editing of RNA transcripts post-transcriptionally.²⁵⁻²⁷ Maxi-circle DNA contains sequences that, when edited, direct the translation of mitochondrial proteins.^{28, 29}

In the vertebrate host, trypanosomes depend entirely upon glucose for energy and are highly aerobic even though the kinetoplast-mitochondrion completely lacks cytochromes. Instead, mitochondrial oxygen consumption is based on an alternative oxidase that does not produce ATP. The parasite develops a conventional cytochrome chain and citric acid cycle in the vector.³⁰

The surface of the trypanosome has numerous membrane-associated transport proteins for obtaining nucleic acid bases, glucose, and other small molecular weight nutrients.^{31, 32, 33} These essential proteins are shielded by allosteric interference from the variant surface glycoproteins (VSGs).³⁴

The trypomastigote enters the bloodstream through the lymphatics and divides further, producing a patent parasitemia. The number of parasites in the blood varies with the stage of disease and whether the infective agent is *T. b. gambiense* (West African trypanosomiasis) or *T. b. rhodesiense* (East African trypanosomiasis). Early in infection with *T. b. rhodesiense* parasites are numerous enough

to be detected on thick blood smears. Blood smears are usually negative in all stages of infection with *T. b. gambiense* due to a lower parasitemia. Trypanosomes can later enter the CNS but remain extracellular, with serious pathological consequences for humans. Some parasites transform into the non-dividing short, stumpy form, which has a biochemistry similar to those of the long, slender form and the form found in the insect vector.³⁵

Both male and female tsetse flies become infected by ingesting a blood meal from an infected host.³⁶ Both male and female tsetse flies take blood meals, unlike mosquitos, where only females take blood meals for egg production.^{37, 38} The short, stumpy forms are pre-adapted to the vector, having a well-developed mitochondrion with a partial citric acid cycle. Meiosis occurs in the parasites immediately following ingestion.³⁹ The trypanosomes then develop into procyclic trypomastigotes in the fly's midgut and divide for approximately ten days. Here, they gain a fully functional cytochrome system and citric acid cycle. When the division cycles are completed, the organisms migrate to the salivary glands and transform into epimastigotes. This form, in turn, divides and transforms further into the metacyclic trypanosome stage and is infectious to humans and reservoir hosts. The cycle in the insect takes 25–50 days, depending upon the species of



Figure 6.4. Impala, one of many reservoirs for *Trypanosoma brucei rhodesiense*.

tsetse, the strain of the trypanosome, and the ambient temperature.³⁶ If tsetse flies ingest more than one trypanosome strain, genetic exchange between the two strains is possible, increasing genetic diversity.⁴⁰

The vector remains infected for life (2–3 weeks for males and up to 4 months for females).⁴¹ Tsetse flies inject over 40,000 metacyclic trypanosomes each time they feed. The minimum infective dose for most hosts is 300–500 organisms, although experimental animals have been infected with a single organism. Infection can also be acquired by eating raw meat from an infected animal.⁴² In East Africa, this mode of transmission may be important in maintaining the cycle in some reservoir hosts, such as lions, cheetahs, leopards, hyenas, and wild dogs.

Cellular and Molecular Pathogenesis

African trypanosomes have lived a balanced coexistence between themselves and non-human hosts since none of the wild animals native to East Africa appear to be severely affected by this parasite (Fig. 6.4). In contrast, humans and the numerous mammals introduced into Africa from Europe, such as non-African breeds of cattle, all suffer the pathological consequences of infection from this group of hemoflagellates. African trypanosomes have evolved several molecu-

lar strategies to avoid elimination from the mammalian host. In addition to antigenic variation, specific genotypes of trypanosomes can survive in the presence of high levels of interferon- γ .⁴³ In contrast, other genotypes can avoid complement-mediated destruction.^{44, 45} Occasionally, some patients are infected with multiple genotypes displaying multiple immune evasion mechanisms.⁴⁶

Infected mammals produce antibodies against a membrane-associated antigen of the trypanosome referred to as the variant surface glycoprotein (VSG).⁴⁷ Specific IgG antibodies destroy all clonal organisms sharing the same surface protein (e.g., VSG-1) by agglutination and lysis. However, some infecting trypanosomes can produce a second variety of surface protein (e.g., VSG-2) with a completely different antigenic signature in addition to the original one. If some of these organisms shed VSG-1 before encountering the antibody and continue to synthesize VSG-2 exclusively, they escape lysis and replace destroyed ones.^{48, 49} A second IgG antibody with specificity to VSG-2 arises, killing all VSG-2 parasites but selecting for VSG-3 organisms, and so on. This antigen-antibody battle between parasite and host continues until the infected individual is overcome by exhaustion due to glucose depletion and the buildup of metabolic wastes from the parasite (Fig. 6.5).

The antigenic variation depends upon the trans-splicing of mRNAs encoded by genes rearranged, duplicated, and expressed at a unique site in the trypanosome genome.⁵⁰

⁵¹ In experimental animal models, the repertoire of antigenic variants of the bloodstream trypomastigotes is extensive, with observed VSGs numbering in the hundreds. The maximum number of VSGs produced in human disease remains unknown, although the genome codes for about 1000. Antigenic variation has presented a challenge for vaccine development against this pathogen.⁵²

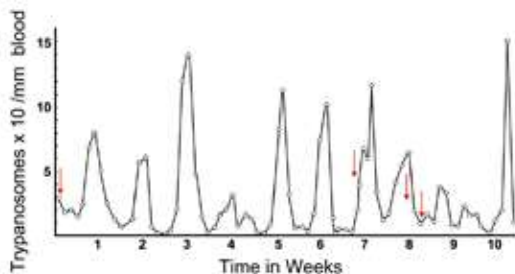


Figure 6.5. Parasitemia in a patient infected with *T. b. rhodesiense*. Each peak of parasitemia represents a new antigenic variant. Arrows indicate attempts at chemotherapy. Ultimately, the patient died of overwhelming infection.

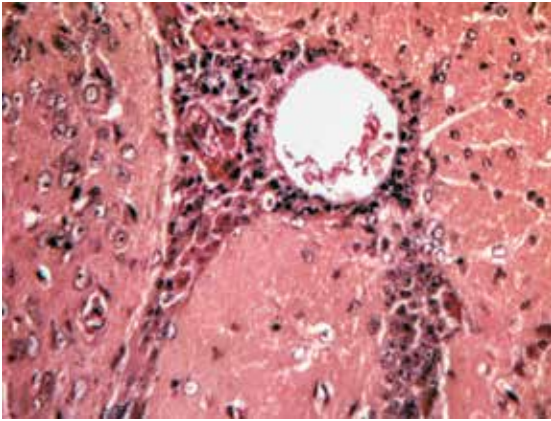


Figure 6.6. Perivascular cuffing around vein in brain of patient who died of sleeping sickness.

Neuropathology

Trypanosomes remain in the bloodstream and lymph nodes throughout the infection period, which can last weeks to years, depending upon the parasite's subspecies and the infected individual's immune capabilities. All nodes become enlarged, but enlargement of the posterior cervical nodes may be the most noticeable.⁵³ This posterior cervical lymph node enlargement is known as "Winterbottom's sign" after Thomas Winterbottom published a report in 1803.⁵⁴ Dr. Winterbottom reported that Arabian slave traders had been aware of this finding and routinely avoided purchasing enslaved people with this finding.⁵⁴ The invasion of the CNS induces a passive condition, leading eventually to coma and death.⁵⁵ Organisms enter the CNS much earlier in the infection with *T. b. rhodesiense* than with *T. b. gambiense*—replication of the parasite in the CSF results in leptomeningitis, cerebral edema, and encephalopathy.⁵⁶ Dysregulated inflammation is the chief pathological correlate, with perivascular cuffing consisting of infiltrates of glial cells, lymphocytes, and plasma cells (Fig. 6.6). Astrocytes are induced to release prostaglandin D₂, a sleep-regulating molecule.⁵⁷ Anti-inflammatory interleukins (interleukin-10 and transforming growth factor- β) are produced early in the infection but lose their effectiveness during the chronic and late phase.⁵⁸

Clinical Disease

The African sleeping sickness caused by the two species of trypanosomes is different in specific ways. A large, painless chancre (Fig. 6.7) containing the dividing organisms usually develops at the bite site within 2–5 days and subsequently heals.¹⁹ The chancre is more often described in *T. b. gambiense* than in *T. b. rhodesiense*. Still, there may be recall bias based on time to diagnosis or a difference in the reaction at the site of the tsetse fly bite due to the differences in host response triggered by the two trypanosomes. Intermittent fever coincides with the organisms entering the bloodstream. Some patients develop rash, generalized pruritus, weight loss, and facial swelling.⁵⁹ Winterbottom's sign is characteristic but not always present.⁵³

Infection rapidly progresses to disease with *T. b. rhodesiense*, with an incubation period of only 2–3 weeks and several weeks. CNS involvement occurs some 3–4 weeks after infection. In contrast, *T. b. gambiense* has an incubation period of several weeks to months and may not involve the brain for months or even years. Europeans exposed to *T. b. gambiense* tend to present with a more rapidly progressive course, suggesting some genetic



Figure 6.7. Chancre due to early infection with *T. b. gambiense*. Courtesy World Health Organization.

differences from African populations.⁶⁰ Two defined stages characterize both forms of sleeping sickness. The early stage is the hemolymphatic stage, and the late stage is the CNS stage. During the early stage, intermittent fever, malaise, and joint pain probably correlate with waves of parasitemia. During this time, generalized enlargement of the lymph nodes occurs, and hepatosplenomegaly may occur.⁶¹ This early stage lasts, on average, for three years with *T. b. gambiense* and for only weeks or months with *T. b. rhodesiense*.⁶²

When trypanosomes invade the CNS, patients experience severe headache, stiff neck, periods of sleeplessness, and depression. Focal seizures, tremors, and palsies are also common. Coma eventually develops, and the patient dies, usually of associated causes such as pneumonia or sepsis. Anemia is a complication of infection with *T. b. rhodesiense* but is not always seen due to the fulminating nature of this form of sleeping sickness.

Diagnosis (see Clinical Appendix)

A history of travel to an endemic area, recalling a painful fly bite, and the presence of a chancre can lead the clinician to the diagnosis. The differential diagnosis includes syphilis, leishmaniasis, and malaria. Given the geographic overlap of the two parasites, co-infection with malaria may occur; however, this should not divert the clinician from diagnosing trypanosomiasis.

Definitive diagnosis depends upon finding the organisms in Wright's or Giemsa-stained blood smears, lymph node aspirates, CSF, or from aspirates taken at the edge of chancres (Fig. 6.3). Cultures are more sensitive than smears since thick blood smears miss a large percentage of infections.⁶³ Several screening programs in endemic areas rely on an initial

serological screening test performed using a card agglutination test for trypanosomiasis.⁶⁴ Antigen detection testing is currently not routinely employed due to concerns about specificity with currently available tests.⁶⁶ Molecular testing is another approach that has been developed, but this approach is still not widely used in endemic areas.^{67, 68} Since parasite concentrations may be low, even in a patient dying of the disease, techniques to improve the sensitivity of diagnosis, such as buffy coat examination and centrifuging sediment of the CSF, have been employed.⁶⁹

Examination of CSF is mandatory in the diagnostic evaluation of trypanosomiasis, and a white blood cell count ≥ 5 cells/ μ L indicates CNS involvement.^{70, 71} Large plasma cells (Mott cells) containing eosinophilic inclusions are uncommon but characteristic and likely represent cells with large amounts of IgM that they cannot secrete.^{72, 73} In the past, determining CNS involvement was essential, as this has guided the choice of appropriate therapy. Today, newer therapies may make this distinction less important.



Figure 6.8. Landsat photograph of African continent, colorized to show vegetation (in green). Photo, NASA.

Treatment (see Clinical Appendix)

Selection of the appropriate treatment for both East African and West African trypanosomiasis may be impacted by the determination of the clinical stage of the disease: early (hemolymphatic stage) versus late (encephalitic stage), and identification of the species of trypanosome.^{74, 75, 76}

For treating West African sleeping sickness (*T. b. gambiense*), a distinction guiding treatment has been first-stage versus second-stage disease, as well as age and weight. Treatment with fexinidazole has been used in individuals with confirmed first-stage or nonsevere second-stage disease.⁷⁷ For individuals < 20 kg and < 6 years of age with confirmed first-stage, one recommended therapy for confirmed early-stage infection has been IV or IM pentamidine.⁷⁸ Individuals with second-stage severe West African sleeping sickness have been treated with combination therapy with nifurtimox and eflornithine. The medication acoziborole, given as a single oral dose, has demonstrated compelling efficacy for both early-stage and late-stage West African Sleeping Sickness and may be the treatment of choice in the future for both early and late-stage disease, avoiding the need for an invasive lumbar puncture.⁷⁹

A distinction guiding treatment for treating East African sleeping sickness (*T. b. rhodesiense*) has been first-stage versus second-stage disease. First-stage disease treatment has been with suramin, which is given even before CSF examination with the suggestion that this may reduce the risk of introduction of parasites into the CSF. In the early hemolymphatic stage of East African sleeping sickness, suramin is the preferred therapeutic agent.^{80, 81} Suramin is only effective in the early stages of the infection as it does not cross the blood-brain barrier.⁷⁴ Melarsoprol has been used for second-stage disease. Unfortunately, melarsoprol has been the only

drug for treating *T. b. rhodesiense* with CNS involvement, and this toxic drug is associated with encephalopathy in about 3% of cases.⁸² The oral medication fexinidazole is being studied to treat East African sleeping sickness. Although Eflornithine is an effective therapy for the late stage of *T. b. gambiense* infection, it is ineffective against *T. b. rhodesiense*.⁸³

Prevention and Control

Previously, periods of political upheaval in different parts of Africa have resulted in dramatic increases in human cases of sleeping sickness.⁸⁴ A 2005 World Health Organization report indicated at least 450,000 new cases that year alone, while before 1995, the estimate was fewer than 70,000.⁸⁵ Military action and civil unrest in the Sudan, Ethiopia, Sierra Leone, Congo, and Liberia have been responsible for the forced migration of millions of individuals, placing them at high risk from several parasitic infections. Tsetse fly control programs have also compromised in these regions due to political and economic instability, exacerbating an already intractable situation. *T. b. gambiense* may exist in a latent state in humans, and activation may play a role in some outbreaks under stress conditions.⁸⁶ In addition to all this turmoil, HIV/AIDS has complicated the picture, adding new dimensions to the general disease control problem. Limited resources in countries bordering conflicted areas cannot keep up with the need for vector control due to large influxes of refugees. Tsetse flies and mosquitoes thrive in disturbed environments.⁸⁷ Despite these challenges, an elimination initiative led by the World Health Organization is underway. Progress has been made, and in recent years, the number of reported cases of HAT is less than 1,000 per year, with over 95% being due to West African trypanosomiasis and about half reported from the DRC.³

Work on vaccines based on VSG antigens

has suggested that highly immunogenic and exposed pathogen-derived antigens may not always be the best target.⁸⁸ Several active investigations continue to advance our understanding of the immune response and what constitutes protection from trypanosomiasis.⁸⁹⁻⁹¹ Other protein antigens, particularly

transporters on the membrane of the flagellar pocket and tubulin, and invariant targets offer promise for human and veterinary vaccines.⁸⁸

⁹² Other than microscopy, diagnostic tests can help in earlier patient diagnosis and control efforts, mainly when there are low parasite numbers and latent infections.

References

1. Simarro, P. P., et al., The Atlas of human African trypanosomiasis: a contribution to global mapping of neglected tropical diseases. *Int J Health Geogr* **2010**, *9*, 57.
2. Simarro, P. P., et al., The human African trypanosomiasis control and surveillance programme of the World Health Organization 2000-2009: the way forward. *PLoS Negl Trop Dis* **2011**, *5* (2), e1007.
3. Papagni, R., et al., Human African Trypanosomiasis (sleeping sickness): Current knowledge and future challenges. *Frontiers in Tropical Diseases* **2023**, *4*.
4. Schares, G.; Mehltitz, D., Sleeping sickness in Zaire: a nested polymerase chain reaction improves the identification of *Trypanosoma* (Trypanozoon) *brucei gambiense* by specific kinetoplast DNA probes. *Tropical medicine & international health : TM & IH* **1996**, *1* (1), 59-70.
5. Migchelsen, S. J., et al., Human African trypanosomiasis: a review of non-endemic cases in the past 20 years. *Int J Infect Dis* **2011**, *15* (8), e517-24.
6. Sinha, A., et al., African trypanosomiasis in two travelers from the United States. *Clin Infect Dis* **1999**, *29* (4), 840-4.
7. Leak, S. G., et al., Use of insecticide-impregnated targets for the control of tsetse flies (*Glossina* spp.) and trypanosomiasis occurring in cattle in an area of south-west Ethiopia with a high prevalence of drug-resistant trypanosomes. *Tropical medicine & international health : TM & IH* **1996**, *1* (5), 599-609.
8. Katakura, K., et al., Detection of *Trypanosoma congolense* and *T. brucei* subspecies in cattle in Zambia by polymerase chain reaction from blood collected on a filter paper. *Parasitology research* **1997**, *83* (3), 241-5.
9. Ghedin, E., et al., Gene synteny and evolution of genome architecture in trypanosomatids. *Molecular and biochemical parasitology* **2004**, *134* (2), 183-91.
10. Berriman, M., et al., The genome of the African trypanosome *Trypanosoma brucei*. *Science* **2005**, *309* (5733), 416-22.
11. Kelly, S., et al., A draft genome for the African crocodilian trypanosome *Trypanosoma grayi*. *Sci Data* **2014**, *1*, 140024.
12. Atkins, J., The Navy Surgeon, or Practical System of Surgery with a Dissertation on Cold and Hot Mineral Springs and Physical Observations on the Coast of Guinea. J. Hodges, at the Looking-Glass, over-against St. Magnus Church, London-Bridge: 1742.
13. Bruce, D., Preliminary Report of the Tsetse Fly Disease or Nagana in Zululand. *Bennet Davis Durban* **1895**.
14. Forde, R. M.; J., Some clinical notes on a European patient in whose blood a trypanosome was observed. *Med* **1902**, *5* 261-263.
15. Stephens, J. W. W., et al., On the peculiar morphology of a trypanosome from a case of sleeping sickness and the possibility of its being a new species (*T. rhodesiense*). *Proc Lond [Biol]* **1910**, *83*, 23-33.
16. Kinghorn, A.; Yorke, W., On the transmission of human trypanosomes by *Glossina morsitans*, and on the occurrence of human trypanosomes in game. *Ann Trop Med Parasitol* **1912**, *6*, 1-23.
17. Texas Southern University. Library.; Heartman Negro Collection., Catalog, Heartman Negro Collection. Library Staff, Texas Southern University: Houston, Texas, 1956; p 325 p.

18. Gull, K., The cell biology of parasitism in *Trypanosoma brucei*: insights and drug targets from genomic approaches? *Current pharmaceutical design* **2002**, 8 (4), 241-56.
19. Naessens, J., et al., Local skin reaction (chancres) induced following inoculation of metacyclic trypanosomes in cattle by tsetse flies is dependent on CD4 T lymphocytes. *Parasite Immunol* **2003**, 25 (8-9), 413-9.
20. Shlomai, J., The structure and replication of kinetoplast DNA. *Current molecular medicine* **2004**, 4 (6), 623-47.
21. Oppendoerfer, F. R.; Borst, P., Localization of nine glycolytic enzymes in a microbody-like organelle in *Trypanosoma brucei*: the glycosome. *FEBS letters* **1977**, 80 (2), 360-4.
22. Verlinde, C. L., et al., Glycolysis as a target for the design of new anti-trypanosome drugs. *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy* **2001**, 4 (1), 50-65.
23. Parsons, M., Glycosomes: parasites and the divergence of peroxisomal purpose. *Molecular microbiology* **2004**, 53 (3), 717-24.
24. Panigrahi, A. K., et al., Identification of novel components of *Trypanosoma brucei* editosomes. *RNA (New York, N.Y.)* **2003**, 9 (4), 484-92.
25. Feagin, J. E.; Stuart, K., Differential expression of mitochondrial genes between life cycle stages of *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences of the United States of America* **1985**, 82 (10), 3380-4.
26. Stuart, K., et al., Investigating the mechanism and machinery of RNA editing. *Methods (San Diego, Calif.)* **1998**, 15 (1), 3-14.
27. Simpson, L., et al., Mitochondrial proteins and complexes in *Leishmania* and *Trypanosoma* involved in U-insertion/deletion RNA editing. *RNA (New York, N.Y.)* **2004**, 10 (2), 159-70.
28. Stuart, K., Mitochondrial DNA of an African trypanosome. *J Cell Biochem* **1983**, 23 (1-4), 13-26.
29. Shapiro, T. A., Kinetoplast DNA maxicircles: networks within networks. *Proceedings of the National Academy of Sciences of the United States of America* **1993**, 90 (16), 7809-13.
30. Walker, R., et al., The effect of over-expression of the alternative oxidase in the procyclic forms of *Trypanosoma brucei*. *Mol Biochem Parasitol* **2005**, 139, 153-62.
31. Borst, P.; Fairlamb, A. H., Surface receptors and transporters of *Trypanosoma brucei*. *Annual review of microbiology* **1998**, 52, 745-78.
32. Sanchez, M. A., et al., Cloning and functional expression of a gene encoding a P1 type nucleoside transporter from *Trypanosoma brucei*. *The Journal of biological chemistry* **1999**, 274 (42), 30244-9.
33. Natto, M. J., et al., *Trypanosoma brucei*: expression of multiple purine transporters prevents the development of allopurinol resistance. *Experimental parasitology* **2005**, 109 (2), 80-6.
34. Donelson, J. E., Antigenic variation and the African trypanosome genome. *Acta tropica* **2003**, 85 (3), 391-404.
35. Kioy, D., et al., Human African trypanosomiasis. *Nature reviews. Microbiology* **2004**, 2 (3), 186-7.
36. Vickerman, K., et al., Biology of African trypanosomes in the tsetse fly. *Biology of the cell / under the auspices of the European Cell Biology Organization* **1988**, 64 (2), 109-19.
37. Channumsin, M., et al., Blood meal analysis of tsetse flies (*Glossina pallidipes*: Glossinidae) reveals higher host fidelity on wild compared with domestic hosts. *Wellcome Open Res* **2021**, 6, 213.
38. Weitz, B., The feeding habits of *Glossina*. *Bull World Health Organ* **1963**, 28 (5-6), 711-29.
39. Peacock, L., et al., Meiosis and haploid gametes in the pathogen *Trypanosoma brucei*. *Curr Biol* **2014**, 24 (2), 181-6.
40. Gibson, W., Genetic exchange in trypanosomes. *Bulletin et memoires de l'Academie royale de medecine de Belgique* **1996**, 151 (2), 203-10.
41. Leak, S. G. A., Tsetse biology and ecology : their role in the epidemiology and control of trypanosomiasis. CABI Publishing in association with International Livestock Research Institute: Oxon ; New York, 1999; p xxiii, 568 p.
42. Betram, B. C. R., Sleeping sickness survey in the Serengeti area (Tanzania) . III. Discussion of the relevance of the trypanosome survey to the biology of large mammals in the Serengeti. *Acta Trop Basel* **1973**, 30, 36-48.

43. Wu, H., et al., Interferon Gamma in African Trypanosome Infections: Friends or Foes? *Frontiers in immunology* **2017**, 8, 1105.
44. Donelson, J. E., et al., Multiple mechanisms of immune evasion by African trypanosomes. *Molecular and biochemical parasitology* **1998**, 91 (1), 51-66.
45. Barry, J. D.; McCulloch, R., Antigenic variation in trypanosomes: enhanced phenotypic variation in a eukaryotic parasite. *Advances in parasitology* **2001**, 49, 1-70.
46. Truc, P., et al., Genetic variability within *Trypanosoma brucei gambiense*: evidence for the circulation of different genotypes in human African trypanosomiasis patients in Cote d'Ivoire. *Trans R Soc Trop Med Hyg* **2002**, 96 (1), 52-5.
47. Cross, G. A., Identification, purification and properties of clone-specific glycoprotein antigens constituting the surface coat of *Trypanosoma brucei*. *Parasitology* **1975**, 71 (3), 393-417.
48. Pays, E., et al., Genetic controls for the expression of surface antigens in African trypanosomes. *Annual review of microbiology* **1994**, 48, 25-52.
49. McCulloch, R., Antigenic variation in African trypanosomes: monitoring progress. *Trends in parasitology* **2004**, 20 (3), 117-21.
50. Gray, A. R., Antigenic variation in a strain of *Trypanosoma brucei* transmitted by *Glossina morsitans* and *G. palpalis*. *Journal of general microbiology* **1965**, 41 (2), 195-214.
51. Borst, P., et al., Control of VSG gene expression sites in *Trypanosoma brucei*. *Molecular and biochemical parasitology* **1998**, 91 (1), 67-76.
52. Barbour, A. G.; Restrepo, B. I., Antigenic variation in vector-borne pathogens. *Emerging infectious diseases* **2000**, 6 (5), 449-57.
53. Winterbottom, T. M., An Account of the Native Africans in the Neighborhood of Sierra Leone. 1803; Vol. 2.
54. Steverding, D., The history of African trypanosomiasis. *Parasit Vectors* **2008**, 1 (1), 3.
55. Mhlanga, J. D., et al., Neurobiology of cerebral malaria and African sleeping sickness. *Brain research bulletin* **1997**, 44 (5), 579-89.
56. Odiit, M., et al., Duration of symptoms and case fatality of sleeping sickness caused by *Trypanosoma brucei rhodesiense* in Tororo, Uganda. *East African medical journal* **1997**, 74 (12), 792-5.
57. Pentreath, V. W., et al., The somnogenic T lymphocyte suppressor prostaglandin D2 is selectively elevated in cerebrospinal fluid of advanced sleeping sickness patients. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1990**, 84 (6), 795-9.
58. Sternberg, J. M., Human African trypanosomiasis: clinical presentation and immune response. *Parasite immunology* **2004**, 26 (11-12), 469-76.
59. Uslan, D. Z., et al., A woman with fever and rash after African safari. *Clin Infect Dis* **2006**, 43 (5), 609, 661-2.
60. Blum, J. A., et al., Human African trypanosomiasis in endemic populations and travellers. *Eur J Clin Microbiol Infect Dis* **2012**, 31 (6), 905-13.
61. Boatin, B. A., et al., Use of symptoms and signs for diagnosis of *Trypanosoma brucei rhodesiense* trypanosomiasis by rural health personnel. *Bull World Health Organ* **1986**, 64 (3), 389-95.
62. Malvy, D.; Chappuis, F., Sleeping sickness. *Clin Microbiol Infect* **2011**, 17 (7), 986-95.
63. Robays, J., et al., The effectiveness of active population screening and treatment for sleeping sickness control in the Democratic Republic of Congo. *Trop Med Int Health* **2004**, 9 (5), 542-50.
64. Chappuis, F., et al., Card agglutination test for trypanosomiasis (CATT) end-dilution titer and cerebrospinal fluid cell count as predictors of human African Trypanosomiasis (*Trypanosoma brucei gambiense*) among serologically suspected individuals in southern Sudan. *Am J Trop Med Hyg* **2004**, 71 (3), 313-7.
65. Inojosa, W. O., et al., Diagnosing human African trypanosomiasis in Angola using a card agglutination test: observational study of active and passive case finding strategies. *Bmj* **2006**, 332 (7556), 1479.
66. Asonganyi, T., et al., A multi-centre evaluation of the card indirect agglutination test for trypanosomiasis (TrypTect CIATT). *Ann Trop Med Parasitol* **1998**, 92 (8), 837-44.
67. Deborggraeve, S., et al., Diagnostic accuracy of PCR in gambiense sleeping sickness diagnosis, staging and post-treatment follow-up: a 2-year longitudinal study. *PLoS Negl Trop Dis* **2011**, 5 (2),

- e972.
68. Becker, S., et al., Real-time PCR for detection of *Trypanosoma brucei* in human blood samples. *Diagnostic microbiology and infectious disease* **2004**, *50* (3), 193-9.
 69. Bailey, J. W.; Smith, D. H., The use of the acridine orange QBC technique in the diagnosis of African trypanosomiasis. *Trans R Soc Trop Med Hyg* **1992**, *86* (6), 630.
 70. Kennedy, P. G., Human African trypanosomiasis of the CNS: current issues and challenges. *J Clin Invest* **2004**, *113* (4), 496-504.
 71. Rodgers, J., Human African trypanosomiasis, chemotherapy and CNS disease. *J Neuroimmunol* **2009**, *211* (1-2), 16-22.
 72. Bain, B. J., Russell bodies and Mott cells. *Am J Hematol* **2009**, *84* (8), 516.
 73. Weinstein, T., et al., Electron microscopy study of Mott and Russell bodies in myeloma cells. *J Submicrosc Cytol* **1987**, *19* (1), 155-9.
 74. Jannin, J.; Cattand, P., Treatment and control of human African trypanosomiasis. *Current opinion in infectious diseases* **2004**, *17* (6), 565-71.
 75. Kennedy, P. G., The continuing problem of human African trypanosomiasis (sleeping sickness). *Ann Neurol* **2008**, *64* (2), 116-26.
 76. H, P. D. K., The Drugs of Sleeping Sickness: Their Mechanisms of Action and Resistance, and a Brief History. *Trop Med Infect Dis* **2020**, *5* (1).
 77. Kande Betu Kumesu, V., et al., Safety and efficacy of oral fexinidazole in children with gambiense human African trypanosomiasis: a multicentre, single-arm, open-label, phase 2-3 trial. *Lancet Glob Health* **2022**, *10* (11), e1665-e1674.
 78. Barrett, M. P., et al., Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. *Br J Pharmacol* **2007**, *152* (8), 1155-71.
 79. Betu Kumesu, V. K., et al., Efficacy and safety of acoziborole in patients with human African trypanosomiasis caused by *Trypanosoma brucei* gambiense: a multicentre, open-label, single-arm, phase 2/3 trial. *Lancet Infect Dis* **2023**, *23* (4), 463-470.
 80. Woo, P.; Soltys, M. A., The effect of suramin on blood and tissue forms of *Trypanosoma brucei* and *Trypanosoma rhodesiense*. *Ann Trop Med Parasitol* **1971**, *65* (4), 465-9.
 81. Arroz, J.; Djedje, M., Suramin and metronidazole in the treatment of *Trypanosoma brucei rhodesiense*. *Trans R Soc Trop Med Hyg* **1988**, *82* (3), 421.
 82. Legros, D., et al., [Therapeutic failure of melarsoprol among patients treated for late stage *T.b. gambiense* human African trypanosomiasis in Uganda]. *Bulletin de la Societe de pathologie exotique (1990)* **1999**, *92* (3), 171-2.
 83. Bacchi, C. J., et al., Parasitic protozoa and polyamines. 1987; p 317-44.
 84. Chretien, J.-P.; Smoak, B. L., African Trypanosomiasis: Changing Epidemiology and Consequences. *Current infectious disease reports* **2005**, *7* (1), 54-60.
 85. WHO World Health Organization estimates for 2005.
 86. Welburn, S. C., et al., Beyond Tsetse - Implications for Research and Control of Human African Trypanosomiasis Epidemics. *Trends Parasitol* **2016**, *32* (3), 230-41.
 87. Molyneux, D. H., Vector-borne parasitic diseases--an overview of recent changes. *International journal for parasitology* **1998**, *28* (6), 927-34.
 88. Magez, S., et al., The History of Anti-Trypanosome Vaccine Development Shows That Highly Immunogenic and Exposed Pathogen-Derived Antigens Are Not Necessarily Good Target Candidates: Enolase and ISG75 as Examples. *Pathogens* **2021**, *10* (8).
 89. Silva, M. S., et al., Trans-sialidase from *Trypanosoma brucei* as a potential target for DNA vaccine development against African trypanosomiasis. *Parasitol Res* **2009**, *105* (5), 1223-9.
 90. La Greca, F.; Magez, S., Vaccination against trypanosomiasis: can it be done or is the trypanosome truly the ultimate immune destroyer and escape artist? *Hum Vaccin* **2011**, *7* (11), 1225-33.
 91. Magez, S., et al., Current status of vaccination against African trypanosomiasis. *Parasitology* **2010**, *137* (14), 2017-27.
 92. Lubega, G. W., et al., Immunization with a tubulin-rich preparation from *Trypanosoma brucei* confers broad protection against African trypanosomiasis. *Experimental parasitology* **2002**, *102* (1), 9-22.



David Bruce, M.D. (1855–1931)

Bruce described the bacteria responsible for causing disease in cattle (*Brucella melitensis*) and correctly identified both the vector (tsetse fly) and the protozoan parasite it transmits to cattle (nagana) as a trypanosome, later named after him (*Trypanosoma brucei*). His discovery soon led to the identification of two other trypanosomes, *Trypanosoma brucei gambiense*, and *T. b. rhodesiense*, as the causative agents of African sleeping sickness in humans.

7. American Trypanosomiasis (Chagas 1909)

Trypanosoma cruzi

Pronunciation: \tri-pan-ə-'sō-mə\ \crew see\

Introduction

Trypanosoma cruzi (\try pano so ma\crew see\)) is the causative agent of American trypanosomiasis, also known as Chagas (\shä-gəs\, \chä-gəs\) disease. It is an intracellular parasite during most of its life cycle, unlike its relatives, the African trypanosomes, that live in the blood and lymph.¹ *T. cruzi* infects many species of mammals native to South and Central America and is vector-borne.²⁻⁵ Insects in the order Hemiptera (true bugs), called “kissing bugs,” are the only known vectors (Figs. 7.1, 38.29).⁶ Chronic infection with this parasite often leads to life-threatening disease. Chagas disease is one of the world’s leading causes of cardiomyopathy.⁷⁻⁹

T. cruzi is found throughout Central and South America, infecting millions of people, and autochthonous human Chagas disease is seen in the United States.¹⁰⁻¹⁴ A health economic assessment indicates that Chagas disease generates billions of dollars in economic losses annually.¹⁵ According to the World Health Organization, Argentina has the most significant number of people living with Chagas disease, followed by Brazil and Mexico. Bolivia has the world’s highest prevalence rate.¹⁶ More than 300,000 individuals in the United States are infected with Chagas disease.¹⁷ Strong evidence suggests that transmission occurs within Texas, where dogs are reservoir hosts.^{13, 14, 18} Through globalization, Chagas disease cases are now found in Southern Europe, Australia, and Japan.¹⁹ As demonstrated by a study in Brazil, the incidence of this disease varies considerably based on location, from 0% in some areas to

above 25% in others.²⁰

Control of Chagas disease requires constant vigilance and has now re-emerged in countries that previously reported that they had eliminated transmission of the disease.²¹ Despite concerted efforts at the clinical level to lower the mortality rate of chronic Chagas disease, acute Chagas disease can have a case fatality rate as high as 5%.²² In individuals who already exhibit signs and symptoms of heart disease, the five-year mortality has been estimated at around 17%.²³

In addition to spreading by the kissing bug (Triatomine), the infection can be transmitted through blood transfusion, bone marrow transplants, organ transplants, transplacentally, and ingesting contaminated beverages.²⁴⁻²⁹ Oral transmission is likely the most frequent mechanism in non-human mammals. Oral transmission to humans of American trypanosomiasis has transformed this disease from one restricted to certain geographic hotspots to one seen in urban outbreaks.³⁰ Contamination is now linked to several outbreaks involving guava juice, sugar cane juice, food, water, soup, and fruit juices.³⁰⁻³² Another important transmission route is vertical transmission of *T. cruzi* infection from mother to child.³³ Rats, dogs, sloths, bats, and various non-human primates are important reservoir hosts, depending upon the region.³⁴⁻³⁹ Transmission takes place



Figure 7.1. Kissing bug nymph, feeding.

in both rural and urban settings. Incidence is highest in children, with the notable exceptions of Brazil and Chile.⁴⁰

Historical Information

In 1909, Carlos Chagas (\shä-gäs\, \chägäs\) observed the infective stage of *T. cruzi* by chance while surveying vectors of malaria.⁴¹

⁴² Carlos Chagas inoculated many species of mammals with the new agent and showed that they all became infected. He correctly speculated that humans were likely to be infected and identified infected people in rural areas of Brazil. He also described the major clinical features of the disease and the morphology of the trypomastigote stage of the parasite. All this work was accomplished within months of his initial discovery. He named the organism after his beloved teacher and close friend, Oswaldo Cruz. Chagas went on to describe the essentials of the life cycle as well. A few years later, in 1912, Alexandre Brumpt completed the description of the life cycle of *T. cruzi*. Then, in 1916, Gaspar Vianna published the details of the pathological consequences of infection with this important pathogenic protozoan.^{42, 43}

For a somewhat gory account of what it's like to wake up covered with well-fed kissing bugs, see Charles Darwin's description in his famous journal, *Voyage of The Beagle*.⁴⁴ Because of this encounter, much speculation has centered on the possibility that Darwin contracted and suffered from chronic Chagas disease. Charles Darwin most likely suffered from lactose intolerance masquerading as Chagas disease!⁴⁵

Life Cycle

The biology, molecular biology, and epidemiology of American trypanosomiasis are starting to be revealed at the genetic level.⁴⁶

⁴⁸ Organisms (metacyclic trypomastigote

stage) are present in the fecal droppings of the infected reduviid bug (Fig. 7.1). The vector ingests a large quantity of blood. To make room for the new meal, it defecates at the time of feeding, depositing feces adjacent to the bite wound. Triatome species in the United States exhibit delayed defecation and movement away from the host after feeding, likely limiting transmission.⁶ The salivary secretions of the bug induce itching, causing the victim to rub the bug feces, laden with parasites, into the wound or mucous membranes.⁶

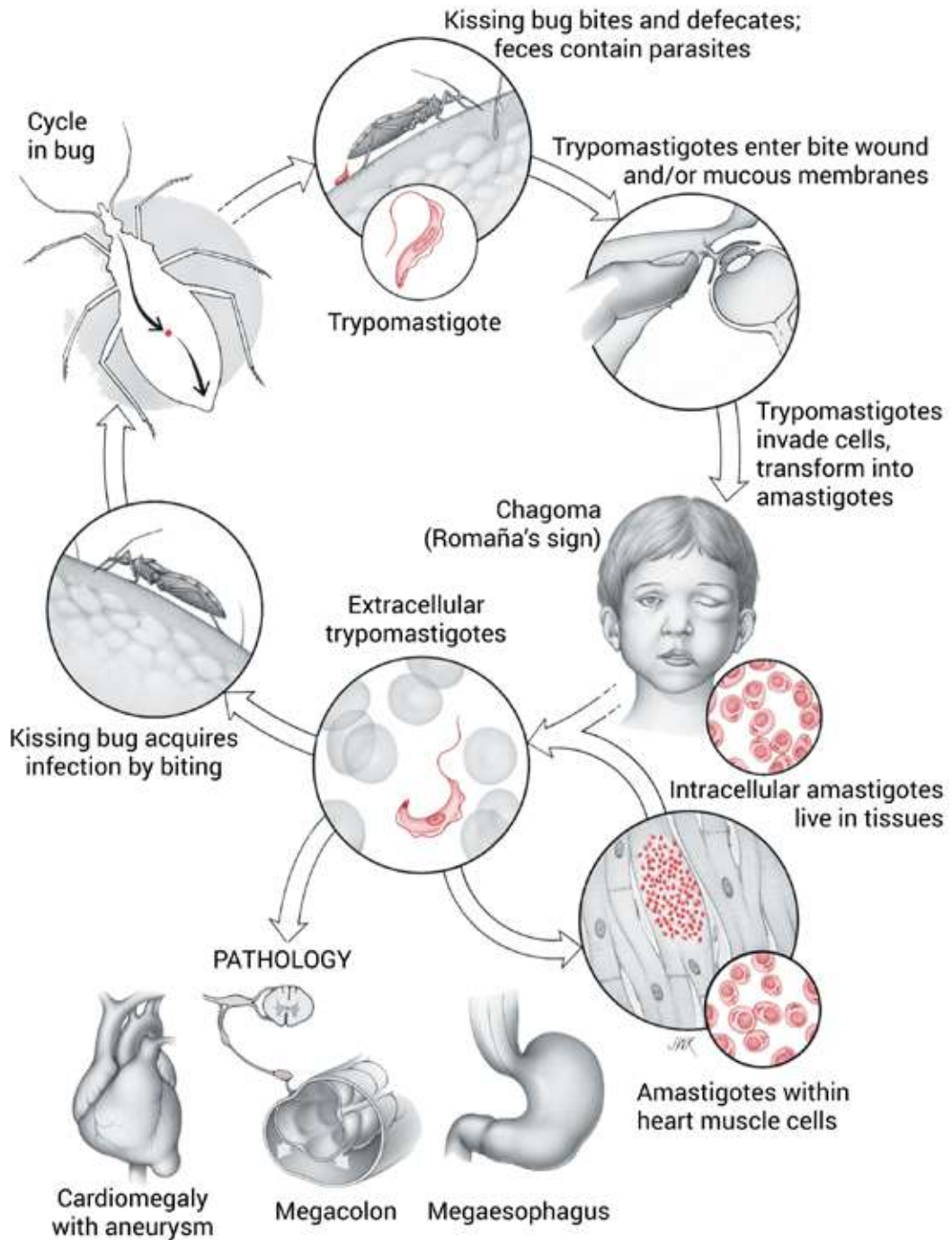
Triatomid bugs are large, robust insects and characteristically feed at night, biting the victim near the mouth or eyes while asleep. The reduviid bug is often called the “kissing bug” because the bite is painless and usually bites around the mouth or eyes.⁴⁹ The bug's saliva contains antigens that induce intense puritis.⁵⁰

The infection can also occur without direct contact with the vector. Oral ingestion is an alternative and well-described means of transmission. Thatched roofs of rural houses can harbor large numbers of bugs (Fig. 7.2), and their feces have the opportunity to fall onto people while the victims are sleeping. Simply rubbing the feces laden with parasites into the mucous membranes of the eye or oral cavity can lead to infection. The probability



Figure 7.2. Thatched roof hut. Ideal breeding sites for kissing bugs.

Trypanosoma cruzi



of infection by this route is high because kissing bugs feed on many mammals, and rural peoples live near their livestock and pets. Infection by transfusion, organ transplantation, or congenital transmission introduces the parasite directly into the host.

Attachment is mediated through galectin-3 on the surface of host cells.⁵¹ The parasite protein that binds to galectin-3 has yet to be identified. The trypomastigote can penetrate a wide variety of cells, and calcium ions mediate the process and at least two parasite membrane proteins: a neuraminidase/trans-sialidase, which binds to sialic acid, and penetrin, which binds to heparin sulfate.^{52, 53} Another protein, gp82, might also be necessary to penetrate gastric epithelium if the metacyclic trypomastigote stage is swallowed.⁵⁴ Just such an outbreak occurred in Santa Catarina, Brazil, involving ingesting sugar cane juice contaminated with at least one infected kissing bug. Animals can become infected by ingesting infected kissing bugs, which might be the usual way to acquire the infection.⁵⁵

After entering the parasitophorous vacuole, the trypomastigote enlists several mechanisms to aid in its survival there. It begins by neutralizing the pH of that intracellular space, thereby escaping the potentially damaging effects of exposure to the active forms of lysosomal enzymes.⁵³ The organism also produces several proteins that offer additional advantages once inside the host cell. Chagasin is a cysteine protease inhibitor necessary for avoiding lysosomal-derived cysteine protease activity. It also ensures that the parasite has the time to differentiate into the amastigote stage.⁵⁶ Cruzipain is thought to play a significant role in helping the parasite avoid being digested once inside the parasitophorous vacuole. Cruzipain also induces the upregulation of host-derived arginase-2, a known inhibitor of apoptosis.⁵⁷ The parasite may be engineering the longevity of its host

cell while at the same time avoiding the ravages of lysosomal digestion.

The parasite then rapidly penetrates the cytosol and differentiates into the amastigote stage. This is the dividing form of *T. cruzi* and the one that inflicts cell damage on the host. After several division cycles, some of the parasites transform back into trypomastigotes. The affected cells die, releasing the parasites into the bloodstream and distributing them throughout the body. They infect cells in many types of tissues, including the CNS, heart muscle, the myenteric plexus, the urogenital tract, and the reticuloendothelial system.

Triatomines become infected by taking a blood meal from an infected individual.⁵⁸ The trypomastigote migrates to the insect's midgut, transforming into the epimastigote and then undergoing many divisions. Thousands of organisms are produced within one insect without significantly affecting it. The triatomines remain infected for life (~1–2 years). Epimastigotes maintain their place in the insect's gut through specific receptor-ligand interactions involving at least one parasite surface glycoprotein and a carbohydrate lectin on the insect's gut cells.⁵⁹ Epimastigotes transform into metacyclic trypomastigotes and migrate to the hindgut, and from there, they are excreted with feces.

Cellular and Molecular Pathogenesis

Infection with *T. cruzi* results in partial immunosuppression that further aids the parasite in remaining inside the host cell for extended periods.^{60, 61} For example, *in vitro* culture of human dendritic cells infected with *T. cruzi* resulted in a dramatic down-regulation of the synthesis of interleukins 6 and 12, TNF- α , HLA-DR, and CD40 and inhibited their maturation into antigen-processing cells.⁶² Parasite-derived calreticulin may also

be important for amastigote survival in the intracellular environment, implicating a central role in calcium trafficking and storage in the parasite's life.⁶³

The release of trypomastigotes into the bloodstream seemingly places them at risk for immune attack since serum antibodies against them can be demonstrated at this time in the infected host. However, *T. cruzi* has evolved to avoid immune targeting. The surface coat of the free-swimming trypomastigote contains a specific complement regulatory protein that binds the complement C3b protein and complement C4b protein components, inhibiting the alternate pathway.⁶⁴

Host protection can develop despite these highly evolved parasite evasion mechanisms. Immunity depends on CD1d involved in antigen presentation and the upregulation of Interleukin-12 for the production of natural killer cells, the protective arm of the immune system most effective against the amastigotes in the tissues.⁶⁵ Parasites are killed by inducing NO synthase and the production of NO.⁶⁶ CD8⁺ T cells with specificities for parasite antigens are essential in maintaining some control of the infection throughout the chronic phase.⁶⁷

Chagas disease manifests in many organs. Infected individuals remain infected for life, and most of the pathological consequences

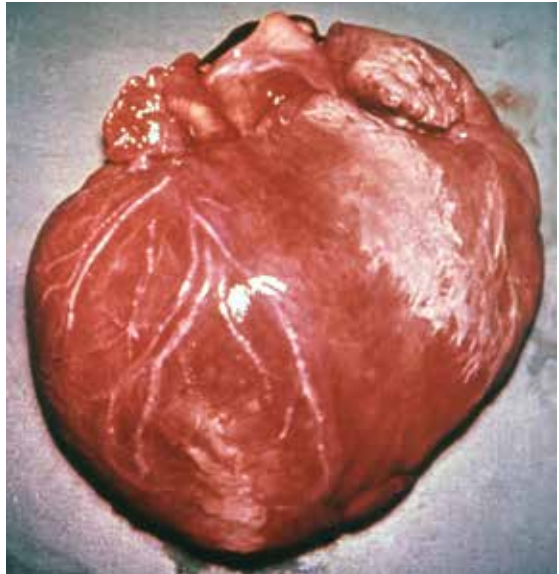


Figure 7.4. Enlarged heart of a patient who died of chronic Chagas disease.

result from cell death (Fig. 7.3). Myenteric plexus damage results in loss of muscle tone and enlargement of the organ, particularly the digestive tract. It is thought that most of the damage to the myenteric plexus directly results from damage that occurs early in infection and is not believed to be prevented by treatment of chronic Chagas.⁶⁸ Megacolon and megaesophagus are late-onset sequelae to chronic illness. Heart damage is almost invariably associated with Chagas disease in some regions of Central and South America and is detectable early in infection.^{69, 70} Erosion of heart tissue is typical and, in many cases, results in aneurysm and heart failure (Figs. 7.4, 7.5).

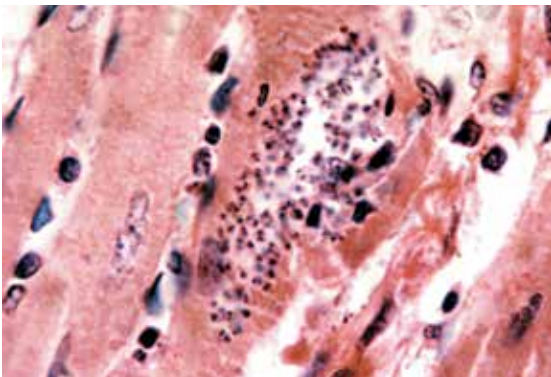


Figure 7.3. Histologic section of heart muscle infected with *Trypanosoma cruzi* amastigotes.



Figure 7.5. Portion of enlarged heart of a patient who died of chronic Chagas disease. Note thin wall of ventricle.

Current thinking regarding a dominant role for auto-antibodies inducing cardiomyopathy plays down this mechanism to account for heart damage during chronic infection.⁷⁰ This is because PCR has demonstrated the presence of *T. cruzi* in heart tissue, even when biopsy material used in conventional histological mode could not reveal the presence of the parasite. Also, the disease progresses rapidly when parasites are abundant and not as fast when they are hard to demonstrate on biopsy.^{70, 71} Thus, parasite persistence leading to fibrosis and damage to the heart (including the cardiac conduction system) has emerged as the dominant theory to explain the pathogenesis of Chagas cardiomyopathy.⁷² Nonetheless, autoantibodies have been detected in many individuals suffering from long-term infection with *T. cruzi*.^{73, 74} Meningoencephalitis can occur during the acute phase and is characterized by infiltrates of CD8⁺ T cells.⁷⁵ Meningoencephalitis can also occur in patients with underlying HIV/AIDS.⁷⁶

Clinical Disease

Acute Chagas Disease

The incubation period of Chagas disease varies based on means of transmission. In acute Chagas disease acquired from the kissing bug, the incubation period is from a few days to two weeks before the onset of symptoms.⁷⁷ With disease acquired through other means, such as transfusion or transplant, the incubation period may be several months.⁷⁸ Acute disease can range from mild nonspecific symptoms such as malaise and fever to severe manifestations. Some patients are asymptomatic. Severe acute manifestations are estimated to develop in approximately 1% of cases and may involve acute myocarditis, pericardial effusion, and, in some instances, meningoencephalitis.^{79, 80} The risk of severe disease and mortality during acute disease tends to be the highest with orally acquired infection.⁸¹

In a few patients, but only the minority, a characteristic hemi-facial edema or swelling called a chagoma develops at the bite site or inoculation within 2–4 days. If organisms are introduced into the body through mucous membranes by rubbing them into the eye, then the swelling associated with the chagoma is known as Romaña's sign.^{82, 83} It occurs mainly as a unilateral swelling. The swollen eyelid is firm to the touch, and conjunctivitis may be associated. If the bite occurs elsewhere, the adjacent area is erythematous, sturdy, and firm to the touch. When the chagoma disappears after several weeks, it leaves an area of depigmentation. An associated neuropathy develops and then disappears when the patient enters the chronic phase of the infection.

Chronic Chagas Disease

Most patients survive the acute phase and become asymptomatic. At this point, they enter the chronic phase, and although they may remain asymptomatic, they can transmit parasites to the insect vector. During the chronic phase of Chagas disease, individuals enter an indeterminant or latent stage, which may continue for the life of most (70–80%) affected individuals or may later manifest with injury to the heart or gastrointestinal system.

The disease progresses in as many as a third of patients, usually as cardiac or gastrointestinal disease.^{84, 85} There continues to be much controversy over the exact mechanism leading to this delayed symptom appearance. Still, there is evidence that parasite persistence may be involved in developing cardiac manifestations.⁸⁴ Another factor is the genetics of the parasite. There are six genetic 'demes' or strains of *T. cruzi*, and certain ones are more prone to cause heart disease than gastrointestinal disease. Ultrastructural studies have demonstrated that vinculin cos-

tameres in cardiomyocytes become disrupted during intracellular infection with the amastigote stage, and this is thought to contribute to cardiomyopathy, which can be seen in chronic infection.⁸⁶

Clinically, the patient experiences extrasystoles, right ventricular enlargement, and eventually heart failure. Right bundle branch block is typically the earliest disturbance evident on ECG, and eventually, the progression of cardiac disease may lead to death.⁸⁷ Some investigators believe that the conduction system disturbances represent the most common cause of death from Chagas-related heart disease rather than the dramatic cardiomyopathy and heart failure seen in some patients.

Gastrointestinal involvement mainly manifests with the development of megaesophagus, characterized by dysphagia, regurgitation, and megacolon, leading to constipation and fecal retention.⁸⁸ The pathogenesis of gastrointestinal disease involves denervation due to the destruction and fibrosis of the submucosal and myenteric nerve plexuses.⁸⁹ The development of gastrointestinal manifestations versus cardiac manifestations may be related to strain differences in the parasite, as the various manifestations differ based on the locales where the infection is acquired, with more gastrointestinal disease seen in individuals acquiring disease in South America rather than Central America.⁹⁰

Disease is not limited to the heart and gut. Rarely, it also leads to megaureters, mega bladders, mega-gallbladders, and bronchiectasis. Adult patients may experience reduced night vision.⁹¹

Reactivation of Chagas disease is now described in patients who become immunocompromised through infection with specific pathogens, such as HIV-1, by taking immu-

nosuppressive medications or after receiving an infected heart transplant.⁹²⁻⁹⁵ Patients with HIV/AIDS exhibit signs and symptoms of the acute phase of infection and, if left untreated, usually die from overwhelming infection due to *T. cruzi* or neurologic involvement.⁷⁶ ⁹⁶ Patients in the chronic phase of infection who acquire HIV can experience a reactivation of *T. cruzi*, resembling the acute phase of the disease.⁹⁷

Congenital Chagas Disease

Congenital Chagas disease is most common in infants born to infected mothers with acute Chagas disease. It has been estimated that 1–10% of infants born to an infected mother will acquire congenital Chagas disease, but the risk appears to be impacted by maternal parasite load.^{98,99} More recent estimates place the vertical transmission rate at around 5%.³³ Transmission can also occur during chronic Chagas disease, as was documented in the United States in August of 2010.¹⁰⁰ Congenital infection can lead to acute disease or may progress to chronic disease without treatment.¹⁰¹

Diagnosis (see Clinical Appendix)

The approach to diagnosis is based on the stage of the disease. In acute disease, the level of parasites in the blood is high enough that trypomastigotes can be detected on exami-



Figure 7.6. Trypomastigote of *T. cruzi*. 20 μ m x 3 μ m.

nation of Giemsa-stained blood smears. A thin and thick smear should be ordered (Fig. 7.6).¹⁰² PCR has greatly facilitated patient diagnosis, allowing earlier detection with improved sensitivity over microscopic evaluation of blood smears.¹⁰²⁻¹⁰⁴

For chronic disease, the diagnosis is usually based on detecting serum IgG.¹⁰⁵ Since no currently available tests for Chagas serology have the required specificity, a diagnosis is based on two positive diagnostic serology tests.¹⁰⁶ It is of note that many patients present to their physician after a positive screening test result when donating blood. This screening test is not diagnostic and would not count as one of the two diagnostic test results in the current Chagas diagnosis paradigm. PCR tests are not routinely employed to diagnose chronic disease. Parasites can also be identified microscopically from biopsy samples of infected tissue. Inoculating blood from suspected individuals into susceptible animals can reveal the organism, but this approach is impractical for most diagnostic facilities. Xenodiagnosis, employing uninfected kissing bugs, allowing them to feed on the patient, and then dissecting the bugs some days later, can also reveal the presence of parasites in chronically infected individuals. It is an involved test requiring extensive laboratory infrastructure and technical assistance. Currently, xenodiagnosis is rarely used.

Treatment (see Clinical Appendix)

The only drugs with proven efficacy against *T. cruzi* are nifurtimox and benznidazole.¹⁰⁶⁻¹⁰⁸ Both drugs are associated with high toxicity and incomplete cure rates in adults, mainly when used to treat the chronic phase of the infection.¹⁰⁸ Benznidazole has been recommended for use in children who have either just acquired the disease (i.e., congenitally) or are in the chronic phase of their infec-

tion.¹⁰⁹ Nifurtimox disrupts *T. cruzi*'s metabolism of carbohydrates, while benznidazole may promote lethal double-stranded DNA breaks in *T. cruzi*.^{110, 111} Both drugs can cause severe side effects, including skin rashes and hematologic disturbances. Also, prolonged treatments are required, lasting 1–2 months, and many patients (up to one in five) cannot tolerate complete treatment courses.

Additional sobering information suggests that it may be too late for the drugs to have a clinical impact once heart involvement begins.¹¹² There is an urgent need to develop improved medicines and clinical algorithms. Newer drugs are being developed, some taking advantage of known metabolic pathways.^{113, 114} Another approach involves the development of a therapeutic vaccine to treat patients in their indeterminant phase to prevent or delay the onset of Chagas cardiomyopathy.¹¹⁵ Such a vaccine could be both cost-effective and cost-saving.¹¹⁶

Heart transplantation as a treatment modality for the cardiomyopathic aspects of Chagas disease has been performed for as long as heart transplantation has been tried in humans.^{117, 118} The fourth recipient ever to receive a heart transplant suffered from chronic *T. cruzi* infection. Current immunosuppressive regimens have allowed for successful transplantation in many patients with cardiac manifestations of Chagas disease.^{117, 118}

Despite a lack of well-controlled trials on the efficacy of treatment for acute Chagas disease, there is a consensus that treatment should be given for both the acute disease and congenitally acquired infection.¹¹⁹ Treatment decisions for chronic Chagas are based on the patient's age and overall health. It is generally recommended that patients with indeterminant stage Chagas, who are under the age of 50, undergo treatment. Treatment for patients over 50 should be individual-

ized based on risks and benefits. Treatment for patients with advanced cardiomyopathy or gastrointestinal manifestations is currently not recommended. Unfortunately, these recommendations for the treatment of chronic disease are not based on well-controlled trials, and a study treating patients with established cardiomyopathy did not show any positive impact on disease progression.¹¹²

Prevention and Control

Control of Chagas disease depends upon interfering with two major transmission routes: vector-borne and transfusion. Control of vectors by prudent use of insecticides (pyrethroids) has significantly reduced transmission of *T. cruzi* in Brazil and Chile.^{120, 121} Unfortunately, this trend has slowly spread to neighboring countries, and there is now evidence of insecticide resistance.¹²² Prevalence remains high throughout Central and South America. Transfusion-induced infection is a problem, especially in countries where *T. cruzi* is not vector-borne, complicating disease control.¹²³ Blood bank screening for *T. cruzi* should be mandatory in all countries experiencing high rates of immigration from South and Central America. In the United States, there is currently selective screening

of blood using serological testing to reduce the chance of transfusion-related transmission of Chagas disease.¹²⁴ It is recommended that paid blood donors be banned in all countries where Chagas disease is endemic.¹²³

A more permanent solution that interfaces well with the concepts of medical ecology is building better housing for people experiencing poverty.^{125, 126} Houses constructed without a thatched roof, slat board wood siding, or rough textured wall surfaces inside the home are relatively safe from kissing bug colonization. Keeping pet dogs and pigs out of the house further reduces the chances of acquiring Chagas disease.¹²⁷

The risk to travelers is considered low, although there have been documented cases of travelers to endemic areas.¹²⁸ It is recommended that travelers sleep under insecticide-treated bed nets and avoid sleeping in dwellings that provide habitats favoring the survival and reproduction of kissing bugs. Consuming sugar cane juice or fruit drinks while on vacation on the beaches of Brazil, Central America, or other parts of South America is likely low-risk despite the occasional outbreaks reported.³⁰⁻³²

References

1. de Souza, W., et al., Review on *Trypanosoma cruzi*: Host Cell Interaction. *Int J Cell Biol* **2010**, 2010.
2. De Araujo, V. A., et al., Mixed infection in the anteater *Tamandua tetradactyla* (Mammalia: Pilosa) from Para State, Brazil: *Trypanosoma cruzi*, *T. rangeli* and *Leishmania infantum*. *Parasitology* **2013**, 140 (4), 455-60.
3. Rocha, F. L., et al., *Trypanosoma cruzi* TcI and TcII transmission among wild carnivores, small mammals and dogs in a conservation unit and surrounding areas, Brazil. *Parasitology* **2013**, 140 (2), 160-70.
4. Rocha, F. L., et al., *Trypanosoma cruzi* infection in neotropical wild carnivores (Mammalia: Carnivora): at the top of the *T. cruzi* transmission chain. *PloS one* **2013**, 8 (7), e67463.
5. Roque, A. L., et al., *Trypanosoma cruzi* among wild and domestic mammals in different areas of the Abaetetuba municipality (Para State, Brazil), an endemic Chagas disease transmission area. *Vet Parasitol* **2013**, 193 (1-3), 71-7.
6. Klotz, S. A., et al., Kissing bugs in the United States: risk for vector-borne disease in humans. *Environ Health Insights* **2014**, 8 (Suppl 2), 49-59.

7. Bocchi, E. A., Heart failure in South America. *Curr Cardiol Rev* **2013**, 9 (2), 147-56.
8. Dokainish, H., et al., Heart Failure in Africa, Asia, the Middle East and South America: The INTER-CHF study. *Int J Cardiol* **2015**, 204, 133-141.
9. Bocchi, E. A., et al., Cardiomyopathy, adult valve disease and heart failure in South America. *Heart* **2009**, 95 (3), 181-9.
10. Schiffier, R. I.; Mansur, G. P., Indigenous Chagas' disease (American trypanosomiasis) in California. *JAMA* **1984**, 251 2983-2984.
11. Woody, N. C.; Woody, H. B., American trypanosomiasis (Chagas' disease): first indigenous case in the USA. *JAMA* **1955**, 159.
12. Global Burden of Disease Study, C., Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **2015**, 386 (9995), 743-800.
13. Marcus, R., et al., Recognition and screening for Chagas disease in the USA. *Ther Adv Infect Dis* **2021**, 8, 20499361211046086.
14. Lynn, M. K., et al., Contemporary autochthonous human Chagas disease in the USA. *Acta Trop* **2020**, 205, 105361.
15. Lee, B. Y., et al., Global economic burden of Chagas disease: a computational simulation model. *Lancet Infect Dis* **2013**, 13 (4), 342-8.
16. Arias-Negrete, S., et al., Expression of *in vitro* virulence by *Entamoeba histolytica*: effect of calmodulin inhibitors. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica* **1999**, 107 (9), 875-81.
17. Hotez, P. J., et al., An unfolding tragedy of Chagas disease in North America. *PLoS Negl Trop Dis* **2013**, 7 (10), e2300.
18. Garcia, M. N., et al., Evidence of autochthonous Chagas disease in southeastern Texas. *Am J Trop Med Hyg* **2015**, 92 (2), 325-30.
19. Schmunis, G. A.; Yadon, Z. E., Chagas disease: a Latin American health problem becoming a world health problem. *Acta Trop* **2010**, 115 (1-2), 14-21.
20. Martins-Melo, F. R., et al., Prevalence of Chagas disease in Brazil: a systematic review and meta-analysis. *Acta Trop* **2014**, 130, 167-74.
21. Reiche, E. M., et al., Seropositivity for anti-*Trypanosoma cruzi* antibodies among blood donors of the "Hospital Universitário Regional do Norte do Paraná", Londrina, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo* **1996**, 38 (3), 233-40.
22. Koberle, F., Chagas' disease and Chagas' syndromes: pathology of American trypanosomiasis. *Adv Parasitol* **1968**, 6, 63-116.
23. Pecoul, B., et al., The BENEFIT Trial: Where Do We Go from Here? *PLoS Negl Trop Dis* **2016**, 10 (2), e0004343.
24. Wendel, S., Transfusion-transmitted Chagas' disease. *Current opinion in hematology* **1998**, 5 (6), 406-11.
25. Blejer, J. L., et al., [Prevalence of *Trypanosoma cruzi* antibodies in blood donors]. *Medicina* **1999**, 59 (2), 129-32.
26. Dictar, M., et al., Recipients and donors of bone marrow transplants suffering from Chagas' disease: management and preemptive therapy of parasitemia. *Bone marrow transplantation* **1998**, 21 (4), 391-3.
27. Carvalho, M. F., et al., Amastigotes forms of *Trypanosoma cruzi* detected in a renal allograft. *Revista do Instituto de Medicina Tropical de Sao Paulo* **1997**, 39 (4), 223-6.
28. Altclas, J., et al., Chagas' disease after bone marrow transplantation. *Bone marrow transplantation* **1996**, 18 (2), 447-8.
29. Russomando, G., et al., Treatment of congenital Chagas' disease diagnosed and followed up by the polymerase chain reaction. *Am J Trop Med Hyg* **1998**, 59 (3), 487-91.
30. Shikanai-Yasuda, M. A.; Carvalho, N. B., Oral transmission of Chagas disease. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2012**, 54 (6), 845-52.
31. Benitez, J. A., et al., Urban outbreak of acute orally acquired Chagas disease in Tachira, Venezuela.

- J Infect Dev Ctries* **2013**, 7 (8), 638-41.
32. Alarcon de Noya, B., et al., Large urban outbreak of orally acquired acute Chagas disease at a school in Caracas, Venezuela. *J Infect Dis* **2010**, 201 (9), 1308-15.
 33. Gebrekristos, H. T.; Buekens, P., Mother-to-Child Transmission of *Trypanosoma cruzi*. *J Pediatric Infect Dis Soc* **2014**, 3 Suppl 1, S36-40.
 34. Steindel, M., et al., Characterization of *Trypanosoma cruzi* isolated from humans, vectors, and animal reservoirs following an outbreak of acute human Chagas disease in Santa Catarina State, Brazil. *Diagn Microbiol Infect Dis* **2008**, 60 (1), 25-32.
 35. Yabsley, M. J., et al., Evaluation of the Chagas Stat-Pak assay for detection of *Trypanosoma cruzi* antibodies in wildlife reservoirs. *J Parasitol* **2009**, 95 (3), 775-7.
 36. Peterson, A. T., et al., Ecologic niche modeling and potential reservoirs for Chagas disease, Mexico. *Emerg Infect Dis* **2002**, 8 (7), 662-7.
 37. Lima, M. M., et al., Investigation of Chagas disease in four periurban areas in northeastern Brazil: epidemiologic survey in man, vectors, non-human hosts and reservoirs. *Trans R Soc Trop Med Hyg* **2012**, 106 (3), 143-9.
 38. Olsen, P. F., et al., Incidence of *Trypanosoma Cruzi* (Chagas) in Wild Vectors and Reservoirs in East-Central Alabama. *J Parasitol* **1964**, 50, 599-603.
 39. Zeledon, R., et al., Wild reservoirs of *Trypanosoma cruzi* with special mention of the opossum, *Didelphis marsupialis*, and its role in the epidemiology of Chagas' disease in an endemic area of Costa Rica. *J Parasitol* **1970**, 56 (1), 38.
 40. Organization, W. H., Chile and Brazil to be certified free of transmission of Chagas' Disease. Tropical Diseases. *Research News (WHO publication)* **1999**, No. 59 June.
 41. Chagas, C., Nova trypanozomiasis humana: estudos sobre a morfologia e o ciclo evolutivo do *Schizotrypanum cruzi*. n genl n sp agente etiológico de nova entidade morbida do homen. *Mem Inst Oswaldo Cruz* **1909**, 1, 159-218.
 42. Brumpt, A. J. E., Le *Trypanosoma cruzi* evolue chez *Conorhinus megistus*. *Cimex ectularius Cimex boueti et Ornithodoros moubata cycle evolutif de ce parasite Bull Soc Pathol Exot* **1912**, 5, 360-364.
 43. Vianna, G., Contribuicao para o estudo da anatomia patologica da anatomia patologica 'moletia de Carlos Chagas' (esquizotripanose humana ou tireodite parasitaria). . *Mem Inst Oswaldo Cruz* **1916**, 3, 276-294.
 44. Darwin, C., The voyage of the Beagle. P.F. Collier & son: New York,, 1909; p 1 p. 1., 547 p.
 45. Campbell, A. K.; Matthews, S. B., Darwin's illness revealed. *Postgraduate medical journal* **2005**, 81 (954), 248-51.
 46. Gull, K., The biology of kinetoplastid parasites: insights and challenges from genomics and post-genomics. *International journal for parasitology* **2001**, 31 (5-6), 443-52.
 47. Miles, M. A., et al., American trypanosomiasis (Chagas' disease) and the role of molecular epidemiology in guiding control strategies. *BMJ (Clinical research ed.)* **2003**, 326 (7404), 1444-8.
 48. Mortara, R. A., et al., Mammalian cell invasion and intracellular trafficking by *Trypanosoma cruzi* infective forms. *Anais da Academia Brasileira de Ciencias* **2005**, 77 (1), 77-94.
 49. Senior, K., Chagas disease: moving towards global elimination. *Lancet Infect Dis* **2007**, 7 (9), 572.
 50. Nichols, N.; Green, T. W., Allergic reactions to "kissing bug" bites. *Calif Med* **1963**, 98, 267-8.
 51. Kleshchenko, Y. Y., et al., Human galectin-3 promotes *Trypanosoma cruzi* adhesion to human coronary artery smooth muscle cells. *Infection and immunity* **2004**, 72 (11), 6717-21.
 52. Ortega-Barria, E.; Pereira, M. E., Entry of *Trypanosoma cruzi* into eukaryotic cells. *Infectious agents and disease* **1992**, 1 (3), 136-45.
 53. Herrera, E. M., et al., Mediation of *Trypanosoma cruzi* invasion by heparan sulfate receptors on host cells and penetrin counter-receptors on the trypanosomes. *Molecular and biochemical parasitology* **1994**, 65 (1), 73-83.
 54. Neira, I., et al., Involvement of *Trypanosoma cruzi* metacyclic trypomastigote surface molecule gp82 in adhesion to gastric mucin and invasion of epithelial cells. *Infection and immunity* **2003**, 71 (1), 557-61.
 55. Calvo Mendez, M. L., et al., Calvo Noguera Alejandro The oral route: an access port for *Trypanosoma cruzi*. *Rev Latinoam Microbiol* **1992**, 34, 39-42.

56. Santos, C. C., et al., Chagasin, the endogenous cysteine-protease inhibitor of *Trypanosoma cruzi*, modulates parasite differentiation and invasion of mammalian cells. *Journal of cell science* **2005**, *118* (Pt 5), 901-15.
57. Aoki, M. P., et al., Cruzipain, a major *Trypanosoma cruzi* antigen, promotes arginase-2 expression and survival of neonatal mouse cardiomyocytes. *Am Cell Physiol* **2003**, *286*, C206-12.
58. Garcia, E. S.; Azambuja, P., Development and interactions of *Trypanosoma cruzi* within the insect vector. *Parasitology today (Personal ed.)* **1991**, *7* (9), 240-4.
59. Pereira, M. E., et al., Lectin receptors as markers for *Trypanosoma cruzi*. Developmental stages and a study of the interaction of wheat germ agglutinin with sialic acid residues on epimastigote cells. *The Journal of experimental medicine* **1980**, *152* (5), 1375-92.
60. Sher, A.; Snary, D., Specific inhibition of the morphogenesis of *Trypanosoma cruzi* by a monoclonal antibody. *Nature London* **1985**, *300*, 639-640.
61. Majumder, S.; Kierszenbaum, F., Mechanisms of *Trypanosoma cruzi*-induced down-regulation of lymphocyte function. Inhibition of transcription and expression of IL-2 receptor gamma (p64IL-2R) and beta (p70IL-2R) chain molecules in activated normal human lymphocytes. *Journal of immunology (Baltimore, Md. : 1950)* **1996**, *156* (10), 3866-74.
62. Van Overtvelt, L., et al., *Trypanosoma cruzi* infects human dendritic cells and prevents their maturation: inhibition of cytokines, HLA-DR, and costimulatory molecules. *Infection and immunity* **1999**, *67* (8), 4033-40.
63. Ferreira, V., et al., Role of calreticulin from parasites in its interaction with vertebrate hosts. *Molecular immunology* **2004**, *40* (17), 1279-91.
64. Beucher, M., et al., Expression and purification of functional, recombinant *Trypanosoma cruzi* complement regulatory protein. *Protein expression and purification* **2003**, *27* (1), 19-26.
65. Duthie, M. S., et al., Both CD1d antigen presentation and interleukin-12 are required to activate natural killer T cells during *Trypanosoma cruzi* infection. *Infection and immunity* **2005**, *73* (3), 1890-4.
66. Vespa, G. N., et al., Nitric oxide is involved in control of *Trypanosoma cruzi*-induced parasitemia and directly kills the parasite in vitro. *Infection and immunity* **1994**, *62* (11), 5177-82.
67. Martin, D. L.; Tarleton, R. L., Antigen-specific T cells maintain an effector memory phenotype during persistent *Trypanosoma cruzi* infection. *Journal of immunology (Baltimore, Md. : 1950)* **2005**, *174* (3), 1594-601.
68. Bern, C., Antitrypanosomal therapy for chronic Chagas' disease. *N Engl J Med* **2011**, *364* (26), 2527-34.
69. Parada, H., et al., Cardiac involvement is a constant finding in acute Chagas' disease: a clinical, parasitological and histopathological study. *International journal of cardiology* **1997**, *60* (1), 49-54.
70. de Andrade, A. L., et al., Early electrocardiographic abnormalities in *Trypanosoma cruzi*-seropositive children. *Am J Trop Med Hyg* **1998**, *59* (4), 530-4.
71. Tarleton, R. L., Chagas disease: a role for autoimmunity? *Trends in parasitology* **2003**, *19* (10), 447-51.
72. Machado, F. S., et al., Pathogenesis of Chagas disease: time to move on. *Front Biosci (Elite Ed)* **2012**, *4*, 1743-58.
73. Vermelho, A. B., et al., Heart muscle cells share common neutral glycosphingolipids with *Trypanosoma cruzi*. *Acta tropica* **1997**, *64* (3-4), 131-43.
74. Cunha-Neto, E., et al., Autoimmunity in Chagas' disease. Identification of cardiac myosin-B13 *Trypanosoma cruzi* protein crossreactive T cell clones in heart lesions of a chronic Chagas' cardiomyopathy patient. *The Journal of clinical investigation* **1996**, *98* (8), 1709-12.
75. Roffe, E., et al., Essential role of VLA-4/VCAM-1 pathway in the establishment of CD8+ T-cell-mediated *Trypanosoma cruzi*-elicited meningoencephalitis. **2003**, *142*, 17-30.
76. Yasukawa, K., et al., *Trypanosoma cruzi* meningoencephalitis in a patient with acquired immunodeficiency syndrome. *Am J Trop Med Hyg* **2014**, *91* (1), 84-5.
77. Anez, N., et al., Update on Chagas' Disease in Venezuela-a review. *Mem Inst Oswaldo Cruz* **2004**, *99*, 781-7.
78. Kun, H., et al., Transmission of *Trypanosoma cruzi* by heart transplantation. *Clin Infect Dis* **2009**,

- 48 (11), 1534-40.
79. Tanowitz, H. B., et al., Perspectives on *Trypanosoma cruzi*-induced heart disease (Chagas disease). *Prog Cardiovasc Dis* **2009**, 51 (6), 524-39.
 80. Andrade, D. V., et al., Acute chagas disease: new global challenges for an old neglected disease. *PLoS Negl Trop Dis* **2014**, 8 (7), e3010.
 81. Beltrao Hde, B., et al., Investigation of two outbreaks of suspected oral transmission of acute Chagas disease in the Amazon region, Para State, Brazil, in 2007. *Trop Doct* **2009**, 39 (4), 231-2.
 82. Dias, J. C., [Cecilio Romana, Romana's sign and Chagas' disease]. *Rev Soc Bras Med Trop* **1997**, 30 (5), 407-13.
 83. Delaporte, F., Romana's sign. *J Hist Biol* **1997**, 30 (3), 357-66.
 84. Gutierrez, F. R., et al., The role of parasite persistence in pathogenesis of Chagas heart disease. *Parasite Immunol* **2009**, 31 (11), 673-85.
 85. Engman, D. M.; Leon, J. S., Pathogenesis of Chagas heart disease: role of autoimmunity. *Acta Trop* **2002**, 81 (2), 123-32.
 86. Melo, T. G., et al., *Trypanosoma cruzi* infection disrupts vinculin costameres in cardiomyocytes. *European journal of cell biology* **2004**, 83 (10), 531-40.
 87. Jorge, M. T., et al., Types of arrhythmia among cases of American trypanosomiasis, compared with those in other cardiology patients. *Annals of tropical medicine and parasitology* **2003**, 97 (2), 139-48.
 88. Meneghelli, U. G., Chagasic enteropathy. *Revista da Sociedade Brasileira de Medicina Tropical* **2004**, 37 (3), 252-60.
 89. Jabari, S., et al., Chagasic megacolon: enteric neurons and related structures. *Histochem Cell Biol* **2014**, 142 (3), 235-44.
 90. Pinazo, M. J., et al., Characterization of digestive involvement in patients with chronic *T. cruzi* infection in Barcelona, Spain. *PLoS Negl Trop Dis* **2014**, 8 (8), e3105.
 91. Matsumoto, S. C., et al., Retinal dysfunction in patients with chronic Chagas' disease is associated to anti-*Trypanosoma cruzi* antibodies that cross-react with rhodopsin. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **2006**, 20 (3), 550-2.
 92. Moreira, M.; Renan Cunha-Melo, J., Chagas Disease Infection Reactivation after Heart Transplant. *Trop Med Infect Dis* **2020**, 5 (3).
 93. Sanchez, A. G., et al., Reactivation of Chagas Disease in Patients With Rheumatic Autoimmune Diseases Diagnosed by Molecular Quantification Techniques. *J Clin Rheumatol* **2021**, 27 (8S), S533-S536.
 94. Inga, L. A. C.; Olivera, M. J., Reactivation of Chagas disease in a heart transplant patient infected by sylvatic *Trypanosoma cruzi* discrete typing unit I. *Rev Soc Bras Med Trop* **2019**, 52, e20180512.
 95. Gray, E. B., et al., Reactivation of Chagas disease among heart transplant recipients in the United States, 2012-2016. *Transpl Infect Dis* **2018**, 20 (6), e12996.
 96. Antunes, A. C. M., et al., Cerebral trypanosomiasis and AIDS. *Arquivos de neuro-psiquiatria* **2002**, 60 (3-B), 730-3.
 97. Harms, G.; Feldmeier, H., The impact of HIV infection on tropical diseases. *Infectious disease clinics of North America* **2005**, 19 (1), 121-35, ix.
 98. Yadon, Z. E.; Schmunis, G. A., Congenital Chagas disease: estimating the potential risk in the United States. *Am J Trop Med Hyg* **2009**, 81 (6), 927-33.
 99. Klein, M. D., et al., Risk factors for vertical transmission of Chagas disease: A systematic review and meta-analysis. *Int J Infect Dis* **2021**, 105, 357-373.
 100. Centers for Disease, C.; Prevention, Congenital transmission of Chagas disease - Virginia, 2010. *MMWR Morb Mortal Wkly Rep* **2012**, 61 (26), 477-9.
 101. Carlier, Y., et al., Congenital Chagas disease: recommendations for diagnosis, treatment and control of newborns, siblings and pregnant women. *PLoS Negl Trop Dis* **2011**, 5 (10), e1250.
 102. Rosenblatt, J. E., Laboratory diagnosis of infections due to blood and tissue parasites. *Clin Infect Dis* **2009**, 49 (7), 1103-8.
 103. Britto, C., et al., Polymerase chain reaction detection of *Trypanosoma cruzi* in human blood samples as a tool for diagnosis and treatment evaluation. *Parasitology* **1995**, 110 (Pt 3), 241-7.

104. Junqueira, A. C., et al., Comparison of the polymerase chain reaction with two classical parasitological methods for the diagnosis of Chagas disease in an endemic region of north-eastern Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1996**, 90 (2), 129-32.
105. Almeida, I. C., et al., A highly sensitive and specific chemiluminescent enzyme-linked immunosorbent assay for diagnosis of active *Trypanosoma cruzi* infection. *Transfusion* **1997**, 37 (8), 850-7.
106. Bern, C., Chagas' Disease. *N Engl J Med* **2015**, 373 (5), 456-66.
107. Kirchhoff, L. V., Changing Epidemiology and Approaches to Therapy for Chagas Disease. *Current infectious disease reports* **2003**, 5 (1), 59-65.
108. Cerecetto, H.; González, M., Chemotherapy of Chagas' disease: status and new developments. *Current topics in medicinal chemistry* **2002**, 2 (11), 1187-213.
109. Schenone, H., et al., [Nifurtimox treatment of chronic Chagasic infection in children]. *Revista medica de Chile* **2003**, 131 (9), 1089-90.
110. Diaz, E. G., et al., Reactions of nifurtimox with critical sulfhydryl-containing biomolecules: their potential toxicological relevance. *Toxicol* **2004**, 24, 189-95.
111. Rajao, M. A., et al., Unveiling benznidazole's mechanism of action through overexpression of DNA repair proteins in *Trypanosoma cruzi*. *Environ Mol Mutagen* **2014**, 55 (4), 309-21.
112. Morillo, C. A., et al., Randomized Trial of Benznidazole for Chronic Chagas' Cardiomyopathy. *N Engl J Med* **2015**, 373 (14), 1295-306.
113. Engel, J. C., et al., Cysteine protease inhibitors cure an experimental *Trypanosoma cruzi* infection. *The Journal of experimental medicine* **1998**, 188 (4), 725-34.
114. Choe, Y., et al., Development of alpha-keto-based inhibitors of cruzain, a cysteine protease implicated in Chagas disease. *Bioorganic & medicinal chemistry* **2005**, 13 (6), 2141-56.
115. Dumonteil, E., et al., Accelerating the development of a therapeutic vaccine for human Chagas disease: rationale and prospects. *Expert Rev Vaccines* **2012**, 11 (9), 1043-55.
116. Lee, B. Y., et al., Modeling the economic value of a Chagas' disease therapeutic vaccine. *Hum Vaccin Immunother* **2012**, 8 (9), 1293-301.
117. de Carvalho, V. B., et al., Heart transplantation in Chagas' disease. 10 years after the initial experience. *Circulation* **1996**, 94 (8), 1815-7.
118. Bocchi, E. A., et al., Heart transplantation for chronic Chagas' heart disease. *The Annals of thoracic surgery* **1996**, 61 (6), 1727-33.
119. Rodrigues Coura, J.; de Castro, S. L., A critical review on Chagas disease chemotherapy. *Mem Inst Oswaldo Cruz* **2002**, 97 (1), 3-24.
120. Schofield, C. J., et al., Advances in Parasitology. 1999; p 2-30.
121. Moncayo, A., Chagas disease: current epidemiological trends after the interruption of vectorial and transfusional transmission in the Southern Cone countries. *Memorias do Instituto Oswaldo Cruz* **2003**, 98 (5), 577-91.
122. Zerba, E. N., Susceptibility and resistance to insecticides of Chagas disease vectors. *Medicina (B Aires)* **1999**, 59 Suppl 2, 41-6.
123. Schmunis, G. A.; Cruz, J. R., Safety of the blood supply in Latin America. *Clinical microbiology reviews* **2005**, 18 (1), 12-29.
124. Agapova, M., et al., Cost-effectiveness of screening the US blood supply for *Trypanosoma cruzi*. *Transfusion* **2010**, 50 (10), 2220-32.
125. Chaudhuri, N., Interventions to improve children's health by improving the housing environment. *Reviews on environmental health* **2004**, 19 (3-4), 197-222.
126. Cecere, M. C., et al., Effects of partial housing improvement and insecticide spraying on the reinfestation dynamics of *Triatoma infestans* in rural northwestern Argentina. *Acta tropica* **2002**, 84 (2), 101-16.
127. Cohen, J. E.; Gürtler, R. E., Modeling household transmission of American trypanosomiasis. *Science (New York, N.Y.)* **2001**, 293 (5530), 694-8.
128. Carter, Y. L., et al., Acute Chagas disease in a returning traveler. *Am J Trop Med Hyg* **2012**, 87 (6), 1038-40.
129. Kroeger, A., et al., Prevention of the transmission of Chagas' disease with pyrethroid-impregnated materials. *Am J Trop Med Hyg* **2003**, 68 (3), 307-11.

8. *Trichomonas vaginalis*

(Donné 1836)

Pronunciation: \tri-kə-'mō-nəs\ \vaj-ə-'nā-ləs\

Introduction

Trichomonas vaginalis (\trick-oh-MOAN-us\ \vaj-gi-NAL-is\) is a flagellated, microaerophilic protozoan mainly transmitted from person to person by sexual contact.^{1, 2} Although non-sexual transmission has been described, it is likely rare.³ Its distribution is worldwide, with high incidence in areas with limited access to healthcare. Prevalence estimates vary widely, and there are significant concerns regarding underdiagnosis and underreporting.⁴⁻⁸ *T. vaginalis* infects both males and females. In males, the infection can be asymptomatic and typically lasts ten days. Most infected females are also asymptomatic, but infection can induce clinical disease, including vaginal itching, inflammation, and purulent discharge, lasting for years if untreated.⁹ Women infected with *T. vaginalis* may experience periods of discomfort, and untreated infection may lead to infertility, pelvic inflammatory disease, cervical neoplasia, and increased susceptibility to HIV-1 infection.¹⁰⁻¹⁴ *T. vaginalis* can also infect the newborn during their passage through the birth canal of an infected woman.¹⁵ Infants may also experience ectopic infection in the respiratory tract and other sites. No reservoir hosts exist, and exposure does not lead to permanent immunity, so reinfection after treatment is common. Although drug-resistant *T. vaginalis* exists, most treatment failures result from noncompliance with therapy.¹⁶ The genome of *T. vaginalis* was sequenced in 2007, and it is very large, with approximately 60,000 protein-coding genes organized into six chromosomes.¹⁷

Historical Information

In 1837, Alfred François Donné described the organism in purulent secretions from women but not the clinical aspects of infection, citing Dujardin's unpublished morphologic description of this flagellate.¹⁸ Over a century later, in 1940, John Kessel and co-workers inoculated healthy volunteers with *T. vaginalis*. Many of these individuals developed the signs and symptoms of the disease. The investigators were then able to match these symptoms with those of naturally infected patients.¹⁹ This study also accurately described the pathologic outcomes of trichomoniasis.

Life Cycle

T. vaginalis exists mainly in the trophozoite stage, measuring 10–25 µm by 7–8 µm (Fig. 8.1). *T. vaginalis* does not produce a classical cyst, but a pseudocyst stage has been recognized that may play a role in cervical neoplasia.^{20, 21} The trophozoite is motile and possesses four flagella projecting from the anterior portion, an undulating membrane

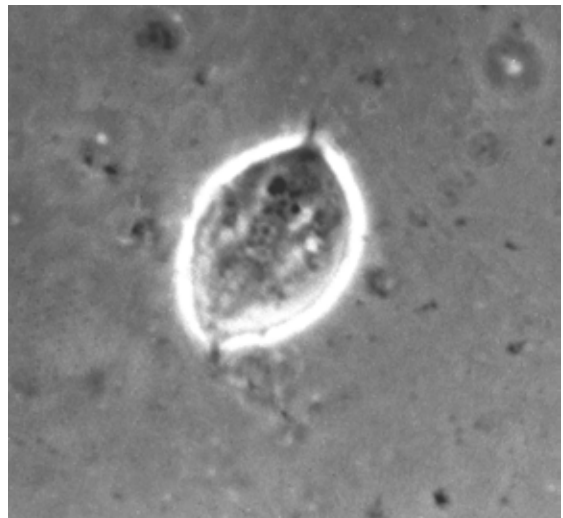
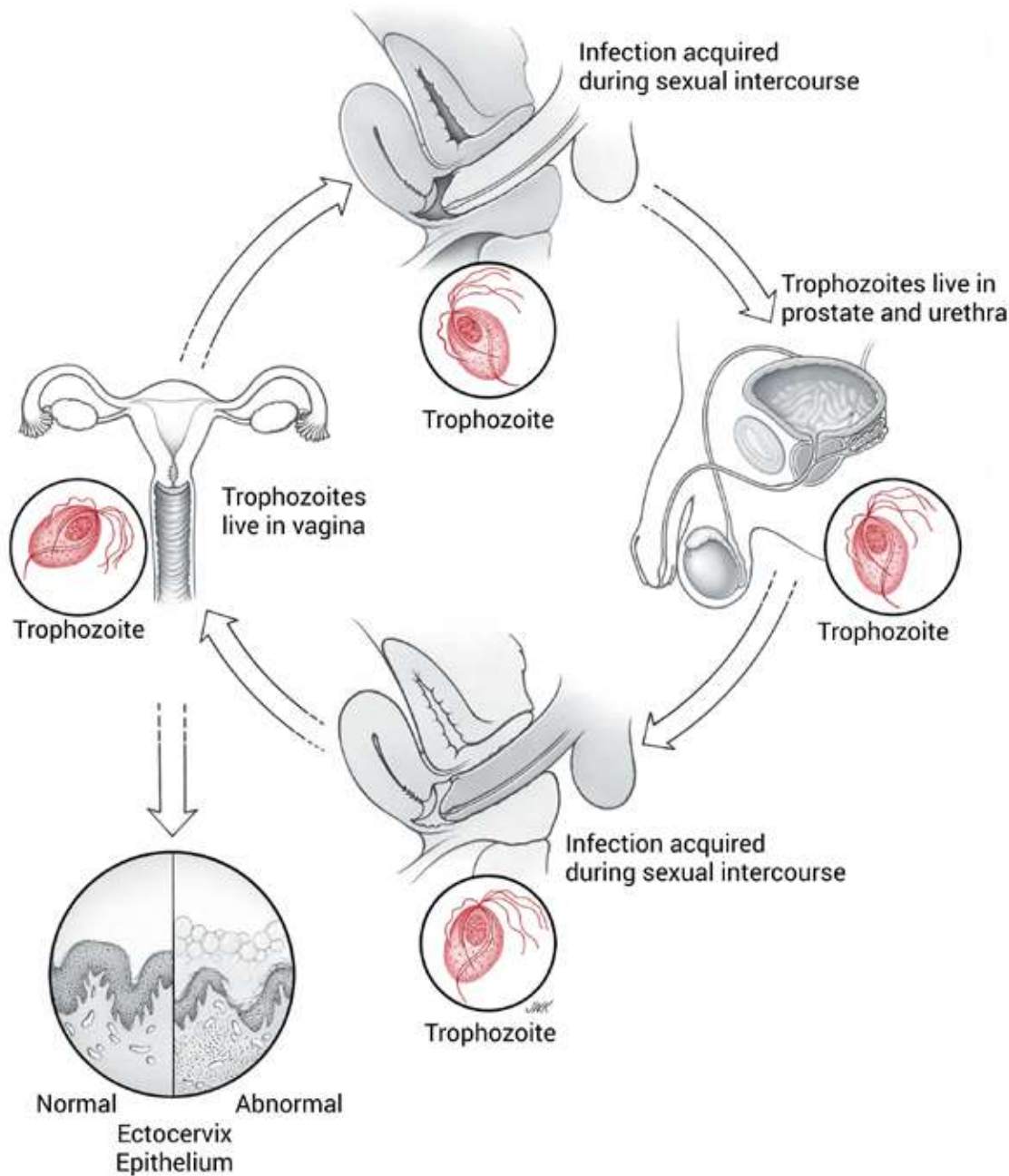


Figure 8.1. Trophozoite of *Trichomonas vaginalis*. Phase contrast. 20 µm x 10 µm.

Trichomonas vaginalis



formed by a posterior extending flagellum, and a rigid axostyle.²² The axostyle is a sheet of microtubules believed to be involved in motility and mitosis.^{23,24}

T. vaginalis has a simple, direct life cycle. The organism is acquired during sexual intercourse with an infected person. *T. vaginalis* then takes up residence in the female urethra, vagina, or endocervix but can spread into the endometrium, adnexa, and Bartholin glands. In men, *T. vaginalis* tends to infect the urethra and prostate. To cause infection, the trophozoites must be able to adhere to epithelial cells. Adhesins and specific ligand-carbohydrate interactions facilitate this.²⁵ Mannose and N-acetyl-glucosamine are two parasite membrane-associated sugar residues used for attachment.^{26, 27} Secretion of lysosomal hydrolases, such as acid phosphatase, occurs at the host-parasite interface immediately following attachment.²⁸ These parasite enzymes are cytotoxic, causing the target cells to lyse and release their contents.²⁹ The parasite then ingests the cell debris. *T. vaginalis* phagocytizes epithelial cells, erythrocytes, and bacteria. The parasite's primary energy source is carbohydrates. *T. vaginalis* uses carbohydrases, including N-acetyl glucosaminidase and α -mannosidase, to detach itself from the target cell membrane and move on to the next cell.³⁰

T. vaginalis reproduces by binary fission, and there is evidence for a sexual cycle, although no morphologically identifiable stage has been described.³¹ Trophozoites secrete molecular hydrogen as a by-product of energy metabolism. Despite its ability to induce clinical disease, *T. vaginalis* is quite fragile and has a limited period of viability, even in moist environments.

Cellular and Molecular Pathogenesis

T. vaginalis possesses an unusual organelle, the hydrogenosome (Figs. 8.2, 8.3), a subcellular organelle derived from an ancient mitochondrion that functions in anaerobic metabolism.³²⁻³⁶ The hydrogenosome contains some necessary enzymes for processing glucose to acetate to molecular hydrogen and putrescine biosynthesis.^{37, 38} The rest of the glycolytic cycle is cytosolic. Putrescine biosynthesis is essential for parasite growth. Inhibition of putrescine synthesis by analogs of putrescine kills the trophozoite.³⁹

Iron plays a vital role in the parasite's attachment process.²⁵ Cell death is the usual outcome because *T. vaginalis* secretes proteases at the attachment site.²⁸ It is not known whether or

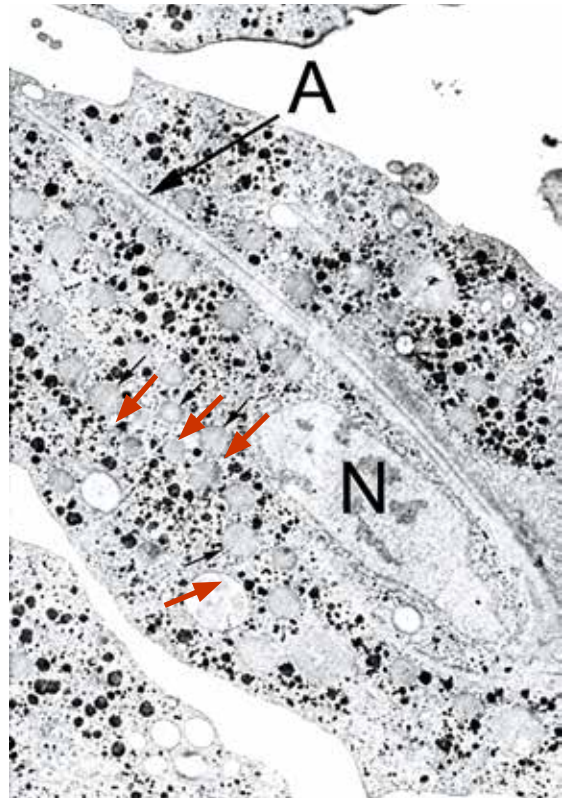


Figure 8.2. Transmission EM of a portion of a trophozoite of *T. vaginalis*. N= nucleus, A= axostyle. Red arrows indicate hydrogenosomes. Courtesy H. Shio.

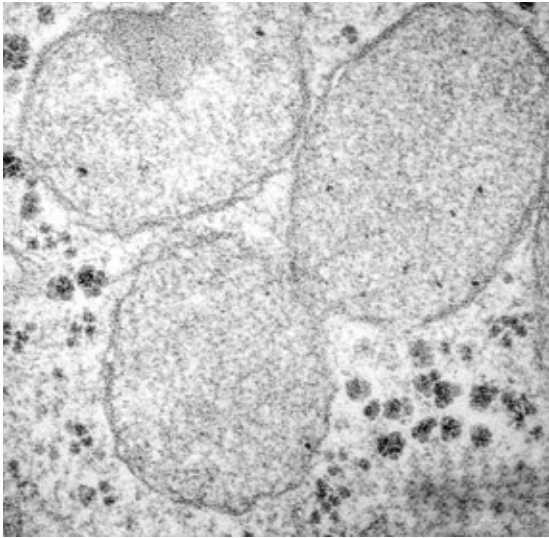


Figure 8.3. Higher magnification EM of hydrogenosomes. Courtesy H. Shio.

not the release of molecular hydrogen into the vaginal tract has any pathological consequence other than producing foul-smelling exudates. Isolates of *T. vaginalis* from patients suffering clinical manifestations showed different capabilities regarding their ability to induce damage in a mouse model. Still, the molecular basis for this variation is not fully understood.⁴⁰ *T. vaginalis* has been divided into two types, type 1 and type 2, based on pathogenicity that may be enhanced by the presence of *T. vaginalis* virus, a double-stranded RNA virus (*Totiviridae*), as well as *Mycoplasma hominis*. In some areas, the majority of isolates show co-infection with these organisms.^{41, 42} The relevance of these co-infections is currently the subject of active investigation.

Clinical Disease

It is estimated that approximately 20% of women infected are symptomatic, while most infected women and men report no significant symptoms. Common clinical symptoms, when present, include mild vaginal discomfort and dyspareunia, vaginal itching, burning on urination associated with thick, yellow, blood-tinged discharge, and rarely

incapacitating illness.^{43, 44} Rarely, urticaria is a complication of heavy infection.⁴⁵ Infection with *T. vaginalis* typically raises the vaginal pH from 4.5 to greater than 5.⁴⁶ Narrow-range pH test strips are best employed to detect this small change. On physical examination, women frequently present with *colpitis macularis* (“strawberry cervix”) and vaginal and vulvar erythema.⁴⁷ All of these signs and symptoms are exacerbated during menstruation.

Symptomatic disease in males can involve the urethra as well as the prostate. Pain in the groin and dysuria may be reported when the prostate becomes infected.⁴⁸ Infection increases the chances of transmission of HIV-1, in part due to disruption of the vaginal wall.^{49, 50}

Infants born of mothers harboring the infection often acquire infection upon passing through the birth canal.¹⁵ Clinical consequences of infection in newborns include urinary tract infection (females only) and rare lung involvement, resulting in a pneumonia-like syndrome.^{51, 52}

Diagnosis (see Clinical Appendix)

The majority of men and women infected with *T. vaginalis* are asymptomatic, with 80–85% of infected females and approximately 80% of infected males reporting no symptoms.^{53,}

⁵⁴ Due to the high prevalence of asymptomatic infection, screening patients rather than relying on symptoms is important, as most *T. vaginalis* infections would be missed. Diagnosis can be made by identifying the organism by microscopic observation, positive culture, rapid antigen testing, nucleic acid probe test, or by use of NAAT (Fig. C.5).⁵⁵ If direct microscopy of wet mount preparations is to be employed, this should be done immediately, as organisms only remain motile for approximately 10 minutes. The sensitivity of

this approach is only about 65%, decreasing with delay.⁵⁶ Culture offers higher sensitivity than wet mount but takes time, limiting its use as a point-of-care test. Rapid antigen testing offers a point-of-care option, and currently, these tests are commercially available but may have variable degrees of sensitivity depending on the test selected.⁵⁷ Nucleic acid probe testing is a highly sensitive option for diagnosis that has demonstrated efficacy for testing vaginal swabs or urine.⁵⁷ Diagnosis by NAAT is far more sensitive than any other method and is now preferred in most hospital parasitology diagnostic laboratories.⁵⁵

Treatment (see Clinical Appendix)

Metronidazole and tinidazole are the drugs of choice.¹ Currently, a 7-day course of metronidazole at 500 mg twice daily is recommended as the preferred option, with a single 2 g dose of tinidazole as an alternative option.⁵⁸ Studies have compared efficacy between HIV-positive and HIV-negative women.^{58, 59} Metronidazole is also available as a vaginal gel but is not recommended for treatment.⁶⁰ Its use as an intra-vaginal suppository did poorly in several clinical trials.⁴⁷ These drugs are typically well tolerated, but metallic taste, antabuse-like side effects with alcohol consumption, and longer-term treatment could have other toxicities.⁶¹ Metronidazole is converted to active intermediates by hydrogenosome-associated pyruvate

ferredoxin oxidoreductase and hydrogenase under anaerobic conditions. The parasite is inhibited from growing by exposure to those intermediates, but the precise biochemical mechanisms of the process are unknown. Resistant strains (approximately 2–5% of all infections) have inactive forms of pyruvate ferredoxin oxidoreductase and hydrogenase, deriving all their energy from glucose by alternate pathways.⁶² Tinidazole, an alternate drug for treating infection, is now available and may be better tolerated as a single 2 g dose option but may be more costly.¹ Reinfection is likely if the infected sexual partner is not treated concurrently. Treatment of the partner, expedited partner therapy (EPT), significantly reduces reinfection, but reinfection is still seen in high-prevalence settings.⁶³ Recurrence may represent reinfection rather than treatment failure, but in refractory cases where drug resistance is suspected, therapeutic options are available, and drug sensitivity testing can be performed.

Prevention and Control

Using a condom during sexual intercourse and limiting the number of sexual partners should reduce the risk of infection. Treating all sexual partners with metronidazole can be effective in some cases, mainly when the number of sexual partners is low. Active screening is essential with the high rate of asymptomatic infections.

References

1. Schwebke, J. R.; Burgess, D., Trichomoniasis. *Clinical microbiology reviews* **2004**, 17 (4), 794-803, table of contents.
2. Leherker, M. W.; Alderete, J. F., Biology of trichomonosis. *Current opinion in infectious diseases* **2000**, 13 (1), 37-45.
3. Crucitti, T., et al., Non-sexual transmission of *Trichomonas vaginalis* in adolescent girls attending school in Ndola, Zambia. *PloS one* **2011**, 6 (1), e16310.
4. Meites, E., et al., *Trichomonas vaginalis* in selected U.S. sexually transmitted disease clinics: testing, screening, and prevalence. *Sex Transm Dis* **2013**, 40 (11), 865-9.
5. Johnston, V. J.; Mabey, D. C., Global epidemiology and control of *Trichomonas vaginalis*. *Curr Opin Infect Dis* **2008**, 21 (1), 56-64.

6. Leitsch, D., Recent Advances in the *Trichomonas vaginalis* field. *F1000Res* **2016**, 5.
7. Takemoto, M. L. S., et al., Prevalence of sexually transmitted infections and bacterial vaginosis among lesbian women: systematic review and recommendations to improve care. *Cad Saude Publica* **2019**, 35 (3), e00118118.
8. Torrone, E. A., et al., Prevalence of sexually transmitted infections and bacterial vaginosis among women in sub-Saharan Africa: An individual participant data meta-analysis of 18 HIV prevention studies. *PLoS Med* **2018**, 15 (2), e1002511.
9. Krieger, J. N., Trichomoniasis in men: old issues and new data. *Sex Transm Dis* **1995**, 22 (2), 83-96.
10. Sayed el-Ahl, S. A., et al., A preliminary study on the relationship between *Trichomonas vaginalis* and cervical cancer in Egyptian women. *J Egypt Soc Parasitol* **2002**, 32 (1), 167-78.
11. Moodley, P., et al., *Trichomonas vaginalis* is associated with pelvic inflammatory disease in women infected with human immunodeficiency virus. *Clin Infect Dis* **2002**, 34 (4), 519-22.
12. El-Shazly, A. M., et al., A study on *Trichomoniasis vaginalis* and female infertility. *J Egypt Soc Parasitol* **2001**, 31 (2), 545-53.
13. Kissinger, P.; Adamski, A., Trichomoniasis and HIV interactions: a review. *Sex Transm Infect* **2013**, 89 (6), 426-33.
14. Chesson, H. W., et al., Estimates of the annual number and cost of new HIV infections among women attributable to trichomoniasis in the United States. *Sex Transm Dis* **2004**, 31 (9), 547-51.
15. Smith, L. M., et al., *Trichomonas vaginalis* infection in a premature newborn. *Journal of perinatology : official journal of the California Perinatal Association* **2002**, 22 (6), 502-3.
16. Kulda, J., Trichomonads, hydrogenosomes and drug resistance. *International journal for parasitology* **1999**, 29 (2), 199-212.
17. Carlton, J. M., et al., Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*. *Science* **2007**, 315 (5809), 207-12.
18. Donne, A.; R., C., Animalcules observes dans la matieres purulentes et le produit des secretions des or-ganes genitaux de l'homme et da la femme. *Seanc Acad Sci* **1837**, 3, 385-386.
19. Kessel, I. F., et al., Observations on the pathology of *Trichomonas vaginitis* and on vaginal implants with *Trichomonas vaginalis* and *Trichomonas intestinalis*. *Am Gynecol* **1940**, 39, 1005-1014.
20. Pereira-Neves, A., et al., Pseudocysts in trichomonads--new insights. *Protist* **2003**, 154 (3-4), 313-29.
21. Afzan, M. Y.; Suresh, K., Pseudocyst forms of *Trichomonas vaginalis* from cervical neoplasia. *Parasitol Res* **2012**, 111 (1), 371-81.
22. Kissinger, P., *Trichomonas vaginalis*: a review of epidemiologic, clinical and treatment issues. *BMC Infect Dis* **2015**, 15, 307.
23. Benchimol, M., et al., The fine structure of the axostyle and its associations with organelles in Trichomonads. *Tissue Cell* **2000**, 32 (2), 178-87.
24. Ribeiro, K. C., et al., Contributions of the axostyle and flagella to closed mitosis in the protists *Tritrichomonas foetus* and *Trichomonas vaginalis*. *J Eukaryot Microbiol* **2000**, 47 (5), 481-92.
25. Garcia, A. F., et al., Iron and contact with host cells induce expression of adhesins on surface of *Trichomonas vaginalis*. *Molecular microbiology* **2003**, 47 (5), 1207-24.
26. Mirhaghani, A.; Warton, A., Involvement of *Trichomonas vaginalis* surface-associated glycoconjugates in the parasite/target cell interaction. A quantitative electron microscopy study. *Parasitology research* **1998**, 84 (5), 374-81.
27. Singh, B. N., et al., Adhesion of *Tritrichomonas foetus* to bovine vaginal epithelial cells. *Infection and immunity* **1999**, 67 (8), 3847-54.
28. Lockwood, B. C., et al., The release of hydrolases from *Trichomonas vaginalis* and *Tritrichomonas foetus*. *Molecular and biochemical parasitology* **1988**, 30 (2), 135-42.
29. Chen, W., et al., Study on ultrastructural cytochemistry and pathogenic mechanism of *Trichomonas vaginalis*. *Chinese medical journal* **1996**, 109 (9), 695-9.
30. Savoia, D.; Martinotti, M. G., Secretory hydrolases of *Trichomonas vaginalis*. *Microbiologica* **1989**, 12 (2), 133-8.

31. Conrad, M. D., et al., Extensive genetic diversity, unique population structure and evidence of genetic exchange in the sexually transmitted parasite *Trichomonas vaginalis*. *PLoS Negl Trop Dis* **2012**, 6 (3), e1573.
32. Embley, T. M.; R., Giezen. M. Hydrogenosomes and mitochondria are two forms of the same fundamental organelle. *Phil Trans Lond* **2002**, 358, 191-201.
33. Vanacova, S., et al., Molecular biology of the amitochondriate parasites, *Giardia intestinalis*, *Entamoeba histolytica* and *Trichomonas vaginalis*. *International journal for parasitology* **2003**, 33 (3), 235-55.
34. PrDiaz, J. A.; Souza, W., De Purification and biochemical characterization of the hydrogenosomes of the flagellate protozoan *Tritrichomonas foetus*. *Eur J Cell Biol* **1997**, 74 (1), 85-91.
35. Williams, B. A. P.; Keeling, P. J., Cryptic organelles in parasitic protists and fungi. *Advances in parasitology* **2003**, 54, 9-68.
36. Lindmark, D. G.; Muller, M., Hydrogenosome, a cytoplasmic organelle of the anaerobic flagellate *Tritrichomonas foetus*, and its role in pyruvate metabolism. *J Biol Chem* **1973**, 248 (22), 7724-8.
37. Wu, G.; Müller, M., Glycogen phosphorylase sequences from the amitochondriate protists, *Trichomonas vaginalis*, *Mastigamoeba balamuthi*, *Entamoeba histolytica* and *Giardia intestinalis*. *The Journal of eukaryotic microbiology* **2003**, 50 (5), 366-72.
38. North, M. J., et al., Polyamine biosynthesis in trichomonads. *Molecular and biochemical parasitology* **1986**, 19 (3), 241-9.
39. Reis, I. A., et al., Inhibition of polyamine synthesis arrests trichomonad growth and induces destruction of hydrogenosomes. *Antimicrobial agents and chemotherapy* **1999**, 43 (8), 1919-23.
40. Hussien, E. M., et al., Biological variability of *Trichomonas vaginalis* clinical isolates from symptomatic and asymptomatic patients. *Journal of the Egyptian Society of Parasitology* **2004**, 34 (3), 979-88.
41. da Luz Becker, D., et al., High rates of double-stranded RNA viruses and *Mycoplasma hominis* in *Trichomonas vaginalis* clinical isolates in South Brazil. *Infect Genet Evol* **2015**, 34, 181-7.
42. Goodman, R. P., et al., Clinical isolates of *Trichomonas vaginalis* concurrently infected by strains of up to four Trichomonasvirus species (Family Totiviridae). *J Virol* **2011**, 85 (9), 4258-70.
43. Deligeoroglou, E., et al., Infections of the lower female genital tract during childhood and adolescence. *Clinical and experimental obstetrics & gynecology* **2004**, 31 (3), 175-8.
44. Petrin, D., et al., Clinical and microbiological aspects of *Trichomonas vaginalis*. *Clinical microbiology reviews* **1998**, 11 (2), 300-17.
45. Purello-D'Ambrosio, F.; Gangemi, S., Urticaria from *Trichomonas vaginalis* infection. *Allergol Clin Immunol* **1999**, 9 (2), 123-5.
46. Petrin, D., et al., Clinical and microbiological aspects of *Trichomonas vaginalis*. *Clin Microbiol Rev* **1998**, 11 (2), 300-17.
47. Wolner-Hanssen, P., et al., Clinical manifestations of vaginal trichomoniasis. *JAMA* **1989**, 26, 571-576.
48. Krieger, J. N.; Riley, D. E., Chronic prostatitis: Charlottesville to Seattle. *The Journal of urology* **2004**, 172 (6 Pt 2), 2557-60.
49. Mason, P. R., et al., Seroepidemiology of *Trichomonas vaginalis* in rural women in Zimbabwe and patterns of association with HIV infection. *Epidemiology and infection* **2005**, 133 (2), 315-23.
50. Moodley, P., et al., *Trichomonas vaginalis* is associated with pelvic inflammatory disease in women infected with human immunodeficiency virus. *Clin Inf Dis* **2002**, 34 (4), 519-22.
51. Hoffman, D. J., et al., Urinary tract infection with *Trichomonas vaginalis* in a premature newborn infant and the development of chronic lung disease. *Journal of perinatology : official journal of the California Perinatal Association* **2003**, 23 (1), 59-61.
52. Szarka, K., et al., Neonatal pneumonia caused by *Trichomonas vaginalis*. *Acta microbiologica et immunologica Hungarica* **2002**, 49 (1), 15-9.
53. Sutton, M., et al., The prevalence of *Trichomonas vaginalis* infection among reproductive-age women in the United States, 2001-2004. *Clin Infect Dis* **2007**, 45 (10), 1319-26.
54. Sena, A. C., et al., *Trichomonas vaginalis* infection in male sexual partners: implications for diagnosis, treatment, and prevention. *Clin Infect Dis* **2007**, 44 (1), 13-22.

55. Negm, A. Y.; el-Haleem, D. A., Detection of trichomoniasis in vaginal specimens by both conventional and modern molecular tools. *Journal of the Egyptian Society of Parasitology* **2004**, *34* (2), 589-600.
56. Krieger, J. N., et al., Diagnosis of trichomoniasis. Comparison of conventional wet-mount examination with cytologic studies, cultures, and monoclonal antibody staining of direct specimens. *JAMA* **1988**, *259* (8), 1223-7.
57. Andrea, S. B.; Chapin, K. C., Comparison of Aptima *Trichomonas vaginalis* transcription-mediated amplification assay and BD affirm VPIII for detection of *T. vaginalis* in symptomatic women: performance parameters and epidemiological implications. *J Clin Microbiol* **2011**, *49* (3), 866-9.
58. Kissinger, P. J., et al., Diagnosis and Management of *Trichomonas vaginalis*: Summary of Evidence Reviewed for the 2021 Centers for Disease Control and Prevention Sexually Transmitted Infections Treatment Guidelines. *Clin Infect Dis* **2022**, *74* (Suppl_2), S152-S161.
59. Howe, K.; Kissinger, P. J., Single-Dose Compared With Multidose Metronidazole for the Treatment of Trichomoniasis in Women: A Meta-Analysis. *Sex Transm Dis* **2017**, *44* (1), 29-34.
60. Workowski, K. A., et al., Sexually Transmitted Infections Treatment Guidelines, 2021. *MMWR Recomm Rep* **2021**, *70* (4), 1-187.
61. Freeman, C. D., et al., Metronidazole. A therapeutic review and update. *Drugs* **1997**, *54* (5), 679-708.
62. Muller, M.; Lossick, J. G., *in vitro* susceptibility of *Trichomonas vaginalis* to metronidazole and treatment outcome in vaginal trichomoniasis. *Sex Trans Dis* **1988**, *15*, 17-24.
63. Lyng, J.; Christensen, J., A double-blind study of the value of treatment with a single dose tinidazole of partners to females with trichomoniasis. *Acta Obstet Gynecol Scand* **1981**, *60* (2), 199-201.



Alexandre Joseph Émil Brumpt, M.D. (1877–1951)

Brumpt was an accomplished zoologist, working on many species of parasites over his lifetime. He published papers on malaria in birds (*Plasmodium gallinaceum*), tsetse fly biology, leishmaniasis, *Trypanosoma cruzi*, *Onchocerca volvulus*, and *Blastocystis hominis*. He also made the distinction between pathogenic *Entamoeba histolytica* and its harmless protozoan doppelganger, *Entamoeba dispar*.

9. The Malarias

Plasmodium

Pronunciation: \plaz-'mō-dē-əm\

P. falciparum

(Welch 1898)

Pronunciation: \fal-'si-pə-rəm\

P. vivax

(Grassi and Filetti 1889)

Pronunciation: \vī-, vaks\

P. ovale

(*P. ovale curtisi* and *P. ovale wallikeri*)
(Stephens 1922)

Pronunciation: \ō-'va-lē\

P. malariae

(Laveran 1880)

Pronunciation: \mə-'lerē, ē\

P. knowlesi

(Knowles and Das Gupta 1932)

Pronunciation: \nōlz ī\

Introduction

Malaria is a mosquito-borne (Fig. 9.1) infection caused by protozoa of the genus *Plasmodium* (\plaz-MODE-ee-um\). Four species of the parasite commonly infect humans: *P. falciparum* (\fal-SIP-pah-rum\), *P. vivax* (\VYE-vax\), *P. ovale* (\OO-va-leh\), and *P. malariae* (\ma-ler-EE-ay\). A fifth species,

P. knowlesi (n-OH-l-z-eye\), has been added to this list of human malaria. *P. knowlesi* is currently restricted to some Southeast Asian regions, with this species responsible for a significant percentage of cases in certain regions.¹ Malaria remains the most important parasitic infection and one of humans' most prevalent infectious diseases.

For much of history, malaria has been a major cause of human morbidity and mortality. In 2017 alone, there were 200 million cases and over 400,000 deaths.²⁻⁶ Following devastating setbacks during the COVID-19 pandemic, the number of cases rose to over 240 million and the number of deaths to over 600,000 per year.⁷ Most of these deaths were among children living in sub-Saharan Africa. Global morbidity and mortality from malaria had been on the decline, and the World Health Organization released a document in 2015 titled: *Global Technical Strategy for Malaria 2016–2030* that, together with other publications, begins the process of considering the technical and financial requirements critical to eliminating malaria as a leading cause of childhood deaths.⁸

Although formerly found throughout much of the world, including the United States, with seasonal outbreaks extending well into temperate zones, malaria is now generally restricted to tropical and subtropical regions.^{9, 10} We are again seeing cases of local transmission of malaria in countries such as the United States, where malaria is no longer endemic.¹¹ This includes the re-emergence of malaria in Florida and Texas and its potential for a resurgence in Southern Europe.^{11, 12} Travel and persistence of the appropriate mosquito species vectors in areas of the world that no longer have the malaria parasites continue to pose the threat of reintroduction into non-immune populations.



Figure 9.1. Adult *Anopheles dirus* taking a blood meal from one of the authors (RWG).

Historical Information

Malaria afflicted humankind's ancestors, as evidenced by its genetic footprint on the human genome.¹³⁻¹⁵ The earliest medical writers in China, Assyria, and India described malaria-like intermittent fevers, which they attributed to evil spirits. By the fifth century BCE, Hippocrates could differentiate quotidian, tertian, and quartan fevers and the clinical symptoms of the disease.¹⁶ At that time, it was assumed that vapors and mists arising from swamps and marshes caused the disease. These theories persisted for more than 2,000 years and were reinforced by repeated observations that the draining of swamps led to a reduction in the number of cases of malaria. Indeed, the names for this disease, malaria (mal, bad; aria, air) and paludism (palus, marsh), reflect these beliefs.

In 1880, concepts surrounding malaria changed with Charles Laveran's description of the crescent-shaped sexual stage of *P. falciparum* and his observation of the dramatic release of the parasite's highly motile microgametes in the fresh blood of an infected soldier.¹⁷ Laveran did not appreciate that there were different *Plasmodium* species. A few years later, in 1886, Camillo Golgi suggested at least two other forms of malaria.¹⁸ Soon afterward, in 1898, Ronald Ross, using a species of bird malaria, and Giovanni Grassi and colleagues working with human malaria showed that the parasite developed in the mosquito and was transmitted by the bite of that insect.¹⁹ Ultimately, Ross and Laveran were awarded Nobel prizes for their contributions.^{20, 21}

Although most of the basic features of the life cycle of the malarial parasite were understood by 1900, it was not until 1947 that Henry Shortt and Cyril Garnham demonstrated in avian malaria that a phase in the liver preceded the parasite cycles in the

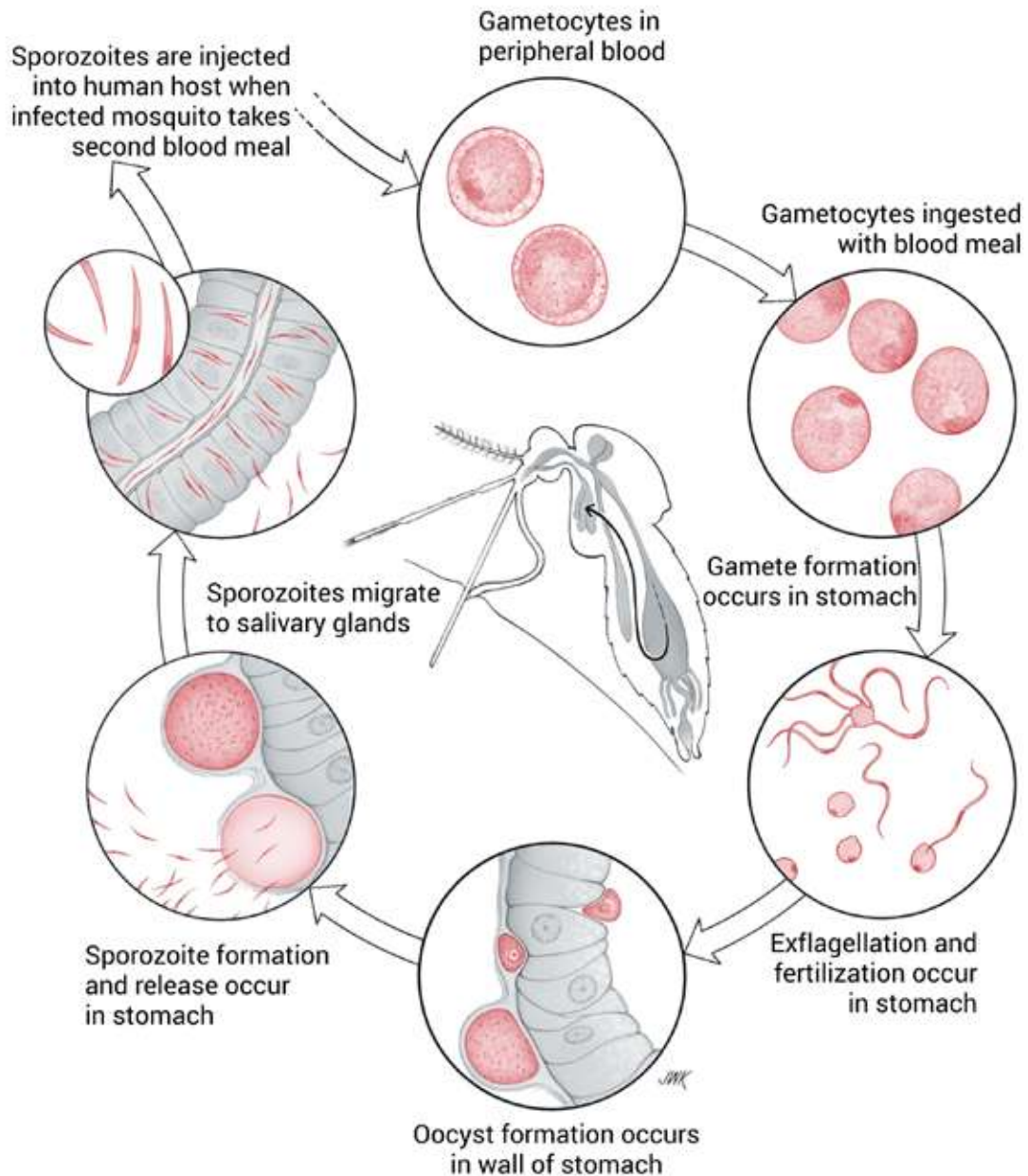


Figure 9.2. Gametocyte of *Plasmodium falciparum*.

blood.²² In 1976, Miles Markus suggested that a dormant stage of *Plasmodium* might exist and suggested the term hypnozoite. It was then in 1980 that Wojciech Krotoski, an American physician, described the dormant liver phase for *P. vivax*.²³⁻²⁶

Early strategies to control malaria mainly sought to reduce the number of mosquitoes and to treat infected people. Chemotherapy of malaria preceded the description of the parasite by nearly 300 years. The Peruvian bark of cinchona, or “fever tree,” was first introduced into Europe in the 1600s.²⁷⁻³⁰ The bark was brought back to Europe by Spanish missionaries and championed by the cardinal Juan de Lugo.³¹ In 1820, Pierre-Joseph Pelletier and Joseph-Bienaimé Caventou isolated the alkaloids of the cinchona tree, quinine, and cinchonine. Synthetic anti-malarial compounds effective against various stages of the parasite were later developed in Germany (pamaquine in 1924, mepacrine in 1930, chloroquine in 1934), in Britain (proguanil in 1944), and in the United States (pyrimethamine and primaquine in 1952).³⁰ Part of the drive for synthetic compounds was the Japanese occupation of Indonesia in 1942, which cut off much of the world from the Dutch plantations there that dominated the industry.³¹

Mosquito Cycle (Sporogony)



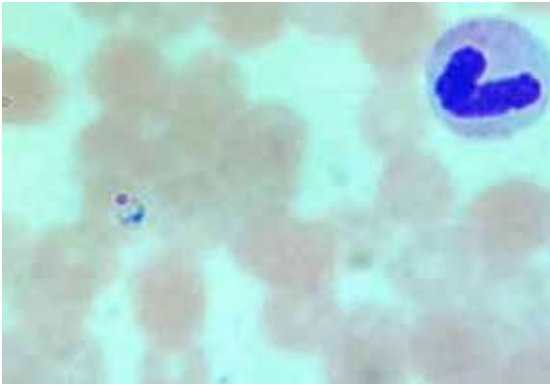


Figure 9.3. Signet ring stage of *Plasmodium* spp.

The Greeks and Romans practiced the earliest forms of malaria control, albeit inadvertently, by draining swamps and marshes. Their primary purpose was the reclamation of land. These techniques were continued for centuries before the role of the mosquito as a vector was discovered. Following the discovery of the mosquito as the vector, malarial control became synonymous with the control of mosquitoes. Destruction of breeding places by drainage and filling the swamps, killing the larvae by placing crude oil on the waters, and later adding the larvicide Paris Green were typical early attempts. With the development of DDT, residual insecticide, large-scale control programs became possible. They culminated in 1957 when the World Health Organization launched a worldwide eradication program, which ultimately failed.

Plasmodium falciparum

Infection caused by *P. falciparum* (Figs. 9.2, 9.16) produces a form of malaria historically referred to as aestivo-autumnal, malignant tertian, or simply *falciparum* malaria. *P. falciparum* is the most pathogenic of human malarias and accounts for most of the mortality from the illness. It is the most prevalent human malarial infection and is mainly confined to tropical and subtropical regions. It is the primary cause of malaria in sub-Saharan Africa.

Identifying *P. falciparum* is usually based on small ring-stage parasites on blood smears (Fig. 9.3). Infected erythrocytes are not enlarged, and multiple infections of single erythrocytes are common. The rings often show two distinct chromatin dots. As trophozoites mature, they become sequestered in the capillaries of internal organs, such as the heart, brain, spleen, skeletal muscles, and placenta, where they complete their development. As a result of sequestration, maturing parasites usually are not present in the peripheral circulation. The appearance of the mature asexual stages (larger trophozoites and schizonts) in the peripheral circulation indicates the increasing severity of the disease.

Gametocytogenesis also proceeds in sequestered erythrocytes and requires approximately ten days. The *falciparum* gametocytes are characteristically crescentic or banana-shaped (Fig. 9.2). They remain infectious for mosquitoes for four days.

Falciparum malaria does not relapse because there is no persistent liver stage (see *P. vivax* and *P. ovale* that do produce hypnozoites). Once parasites have developed to the erythrocytic stage and exit from the hepatocytes, they cannot re-infect the liver. Recrudescence (reappearance of infected erythrocytes from the deep tissues into the peripheral blood) can



Figure 9.4. Trophozoite of *P. vivax*. Schüffner's dots on the surface of the infected red blood cell. The surrounding red cells are smaller than the infected one.

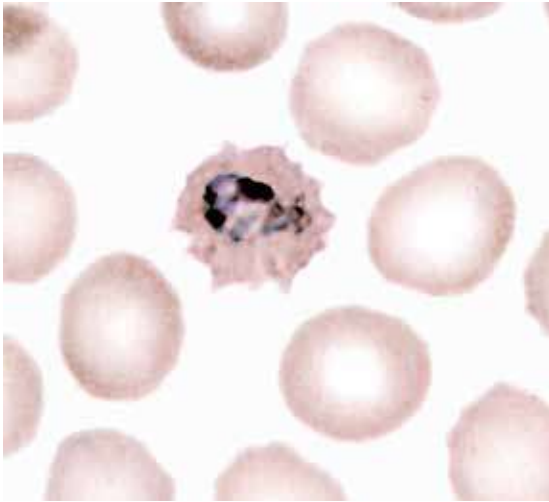


Figure 9.5. Trophozoite of *P. ovale*. Note “crenated” appearance of infected red cell. Courtesy M. Guelpe.

occur soon after recovery or years later, even with treatment.

Plasmodium vivax

P. vivax infection is called benign tertian or vivax malaria. The term *benign* is only relative to falciparum malaria, as there are tens of thousands of deaths every year caused by *P. vivax*.³² Red blood cells infected with *P. vivax* (Fig. 9.4, 9.17) are enlarged and, when adequately stained with Giemsa, often show stippling on the erythrocyte membrane, known as Schüffner’s dots. All stages of the parasite are present in the peripheral circulation. Single infections of invaded erythrocytes are charac-

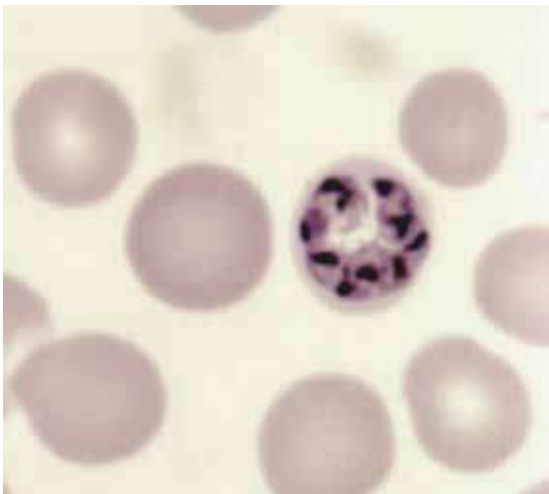


Figure 9.6. Schizont of *P. malariae*. Note uninfected red cells are the same size as the infected cell.

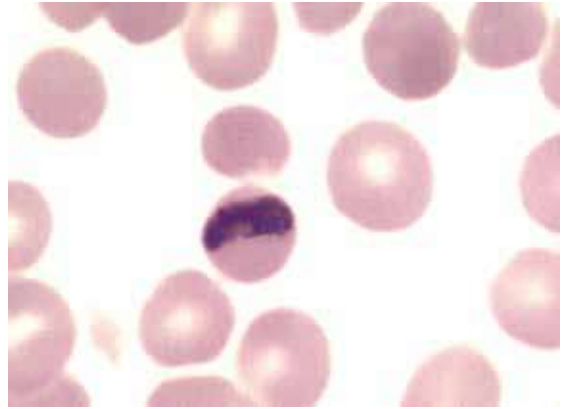


Figure 9.7. *Plasmodium malariae* trophozoite.

teristic. Gametocytes appear simultaneously with the first asexual parasites. The duration of the viability of the sexual stages is less than 12 hours. *P. vivax* produces the classic relapsing malaria, initiated from hypnozoites in the liver after a latency period. Relapses can occur every few weeks to a few months for up to five years after the initial infection. The specific periodicity of the relapses is a characteristic of the geographic strain of the parasite. Vivax malaria also recrudesces due to persistent low numbers of circulating infected erythrocytes.

Plasmodium ovale

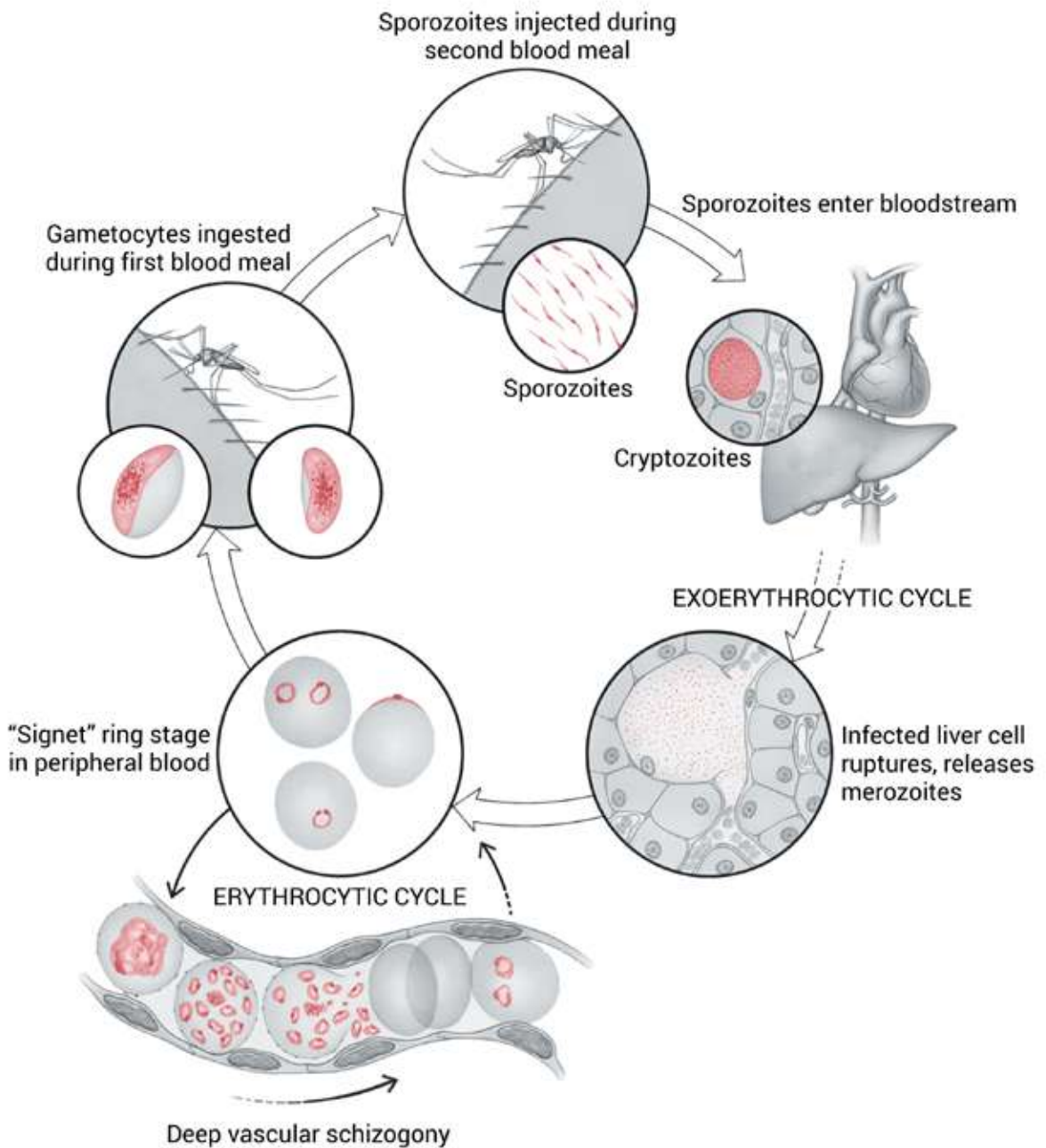
(*P. ovale curtisi* and *P. ovale wallikeri*)

P. ovale (Figs. 9.5, 19.19) is limited to tropical Africa and discrete areas of the Western Pacific. *Ovale* malaria produces a tertian fever clinically similar to vivax malaria but somewhat less severe. It exhibits relapses for the same duration as vivax malaria. It is now known that *P. ovale* is composed of two genetically distinct species that diverged more than 1 million years ago, *P. ovale curtisi* (classic strain) and *P. ovale wallikeri* (variant strain).³³ *P. ovale wallikeri* appears to have a shorter latency period than *P. ovale curtisi*.³⁴

Plasmodium malariae

The disease caused by *P. malariae* is known as quartan malaria. *P. malariae* has a wide but spotty distribution throughout the world. Development in the mosquito is slow, and

Plasmodium falciparum



Plasmodium vivax

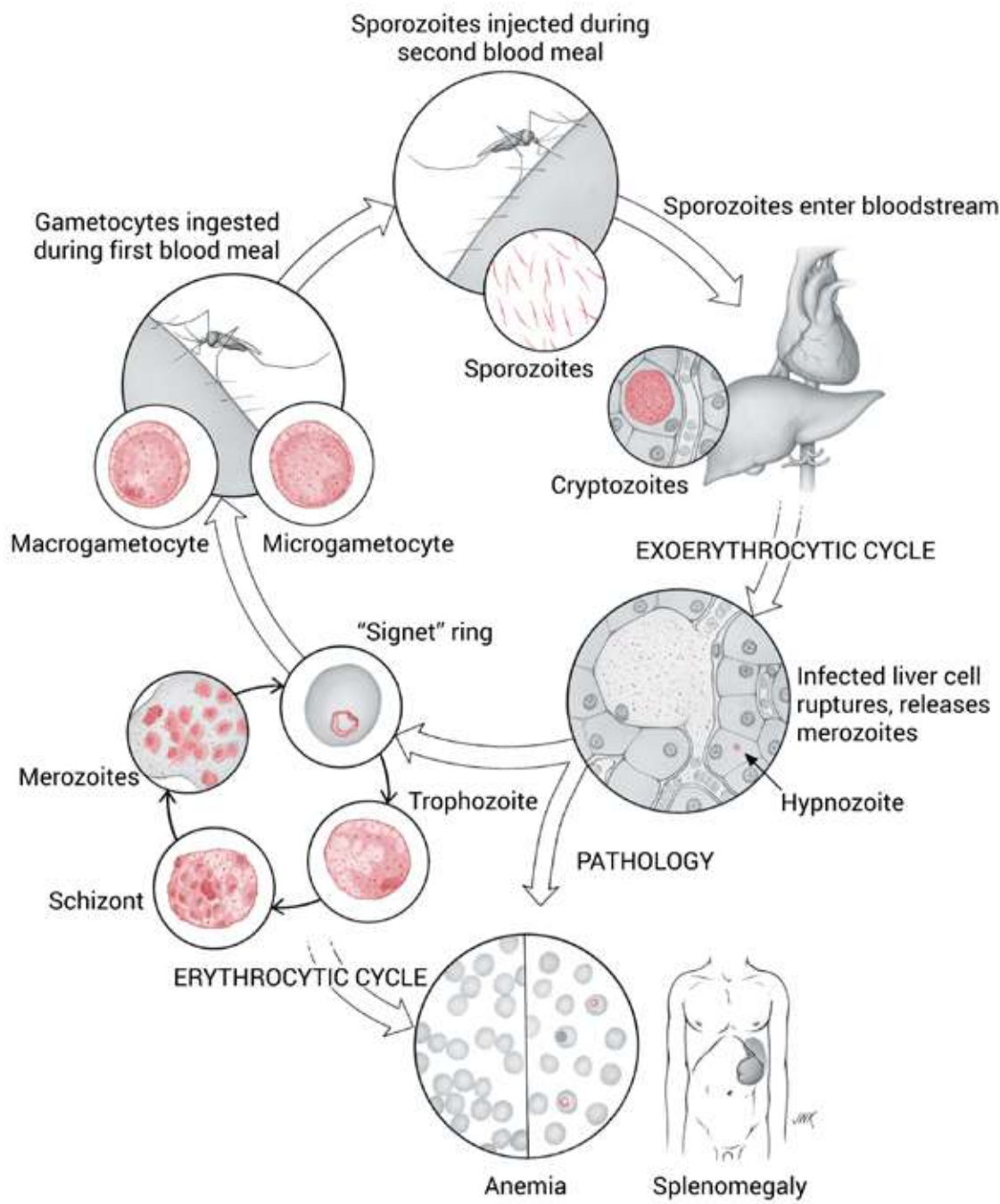




Figure 9.8. Sporozoites of malaria in infected mosquito stomach preparation.

infection in humans is not as intense as that caused by the other *Plasmodium* species. Most current evidence indicates that *P. malariae* does not relapse. It does recrudescence due to chronic low-level parasitemia that may persist for decades.^{35, 36} Erythrocytes infected with *P. malariae* remain the same size throughout schizogony (Figs. 9.6, 9.7, 9.18).

Plasmodium knowlesi

Some species of *Plasmodium* that are parasites of chimpanzees, orangutans, and mon-

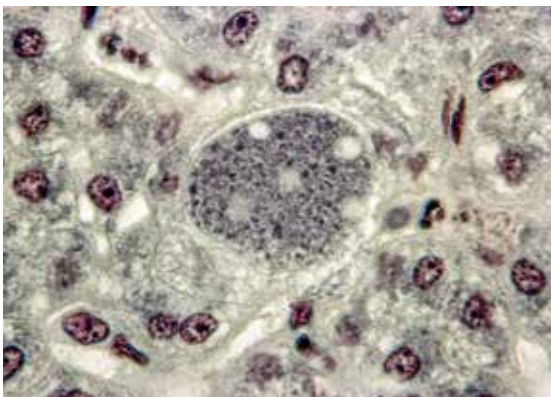


Figure 9.9. Exoerythrocytic stages of malaria in liver parenchymal cell.

keys occasionally infect humans.²¹ In 1932, Knowles and Das Gupta described the experimental transmission of *P. knowlesi* to humans.³⁷ It later became established as the fifth human malaria.^{38, 39} *P. knowlesi* was considered restricted to limited areas of Southeast Asia. Still, cases are being diagnosed in more areas of Southeast Asia, either through increased awareness or an expansion of the geographic range.^{1, 40} Recognition of human infection with *P. knowlesi* may also be due, in part, to our ability to differentiate otherwise morphologically similar human and simian parasites at the molecular level.^{41–43} The disease it causes can range from mild to severe. Most notable is the quotidian fever (24-hour cycle). Certain other simian malaras, such as *P. cynomolgi*, *P. brasilianum*, *P. eylesi*, *P. inui*, *P. schwetzi*, and *P. simium*, may be transmissible to humans through mosquito bites.^{44–46}

Life Cycles

The biology of all *Plasmodium* species is generally similar and consists of two discrete phases: asexual and sexual. The asexual stages develop in humans and other primates, first in the liver and then in the circulating erythrocytes. The sexual stages develop in the mosquito.

Asexual Stages

When the infected female *Anopheles* mosquito takes a blood meal (Fig. 9.1), she injects salivary fluids into the wound. These fluids contain an estimated 150–200 sporozoites (Fig. 9.8), small (10–15 μm long), spindle-shaped, motile forms of the parasite, which initiate the infection.⁴⁷ For many years, it was suggested that if the injected sporozoites had not left the injection site within one hour, it was likely that they would stay there. Work demonstrating that about 90% of injected sporozoites remain at the injection site and that removal of the injection site inoculum

reduced the parasites reaching the liver by over 90% challenged the idea that sporozoites had only minutes to reach the liver.⁴⁸⁻⁵⁰ The timing of the transit from inoculation to infection of hepatic cells and how sporozoites traffic to and enter the liver has been the subject of much investigation.⁵¹⁻⁵³ Evidence suggests that sporozoites bind to CD68 on the surface of Kupffer cells, facilitating entry into hepatocytes.⁵⁴ Once inside the liver cell, the parasites undergo asexual division (exoerythrocytic schizogony; Fig. 9.9). A prescribed number of merozoites are produced over days to weeks, depending upon the species. *P. vivax* matures within 6–8 days, and each sporozoite produces about 10,000 daughter parasites. For *P. ovale*, these values are nine days and 15,000 merozoites; for *P. malariae*, 12–16 days and 2000 merozoites; and for *P. falciparum*, 5–7 days and 40,000 merozoites.⁵⁵

The phenomenon of relapse in certain malarias (*P. vivax*, *P. ovale*, and *P. cynomolgi*) is due to the production of hypnozoites, a latent

liver stage. A parasitologic malarial relapse is the reappearance of parasitemia in peripheral blood in a sporozoite-induced infection following adequate blood schizonticidal therapy.⁵⁶ It had long been accepted that the exoerythrocytic forms of relapsing malaria persist in the liver due to cyclic development (rupture of infected cells and invasion of new cells).⁵⁷ However, experimental evidence has lent support to a different hypothesis for the mechanism of relapse. It holds that some sporozoites fail to initiate immediate exoerythrocytic development in the liver and remain latent, capable of delayed development and initiation of relapse.⁵⁸

Several patterns of relapse have been described, often related to the geographic origin of the parasite. Temperate strains of *P. vivax* may show delayed primary attacks and relapses, whereas more tropical forms emerge from the liver within weeks of infection. In *P. vivax* and *P. ovale* malarias, eradicating parasites from the peripheral circulation with drugs aborts the acute illness. Subsequently, a fresh wave of exoerythrocytic merozoites from the liver can reinstate the infection. The dormant parasites, or hypnozoites, can remain quiescent in the liver for up to five years.^{36,59-61} To achieve a radical cure, destroying the circulating parasites and the



Figure 9.10. Transmission EM of a merozoite entering a red cell. Note points of attachment. Courtesy S. Langreth.

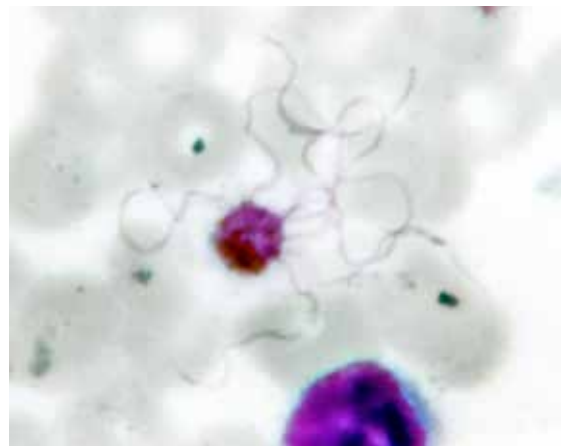


Figure 9.11. Exflagellation of the microgametocyte of a malaria parasite. Each “flagella” is actually a male gamete.

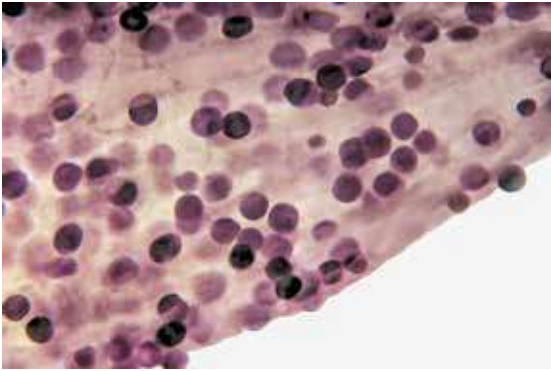


Figure 9.12. Portion of an infected mosquito stomach. Note numerous oocysts on outer wall.

hypnozoites is necessary.⁶²

P. falciparum and *P. malariae* do not develop hypnozoites. They, therefore, cannot relapse. For infections with *P. falciparum* and *P. malariae*, drugs that only eradicate parasites in the peripheral circulation achieve a cure. Untreated *P. falciparum* can recrudesce for 1–2 years by continuing the erythrocytic cycle, which can remain at a subclinical, asymptomatic level for long periods of time. *P. malariae* can recrudesce for 30 years or more.³⁵

Erythrocytic Phase

When merozoites are released from the liver, they invade red blood cells (Fig. 9.10) and initiate the erythrocytic phase of infection. Invasion of the erythrocytes consists

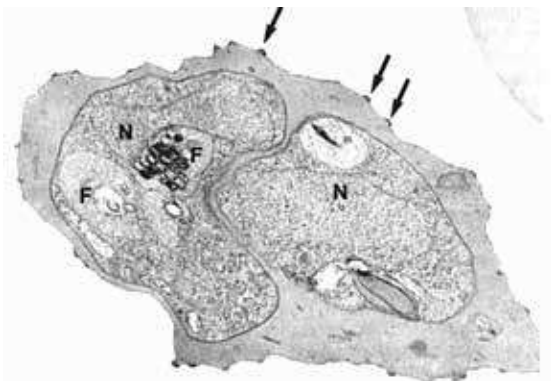


Figure 9.13. Transmission EM of red cell infected with *P. falciparum*. Arrows indicate (“knobs”) points of attachment to host endothelial cells. N=nucleus, F=food vacuole. Courtesy S. Langreth.

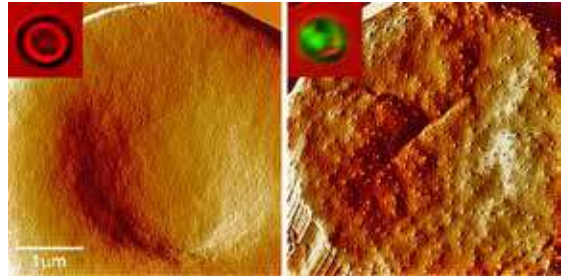


Figure 9.14. Atomic force microscopy of normal (left) and *Plasmodium falciparum*-infected (right) red cells. Courtesy J. Dvorak.

of a complex sequence of events, beginning with contact between a free-floating merozoite and the red blood cell.⁶³ Attachment of the merozoite to the erythrocyte membrane involves interaction between *Plasmodium* binding proteins and specific receptors on the surface of erythrocytes.⁶⁴ For example, transferrin receptor 1 appears to be the receptor for *P. vivax* reticulocyte binding protein 2b.⁶⁵ After that, the erythrocyte undergoes rapid and marked deformation. The parasite enters by a localized endocytic invagination of the red blood cell membrane, utilizing a moving junction between the parasite and the host cell membrane.⁶⁶



Figure 9.15. Child infected with malaria, probably *P. malariae*. Note enlarged spleen.

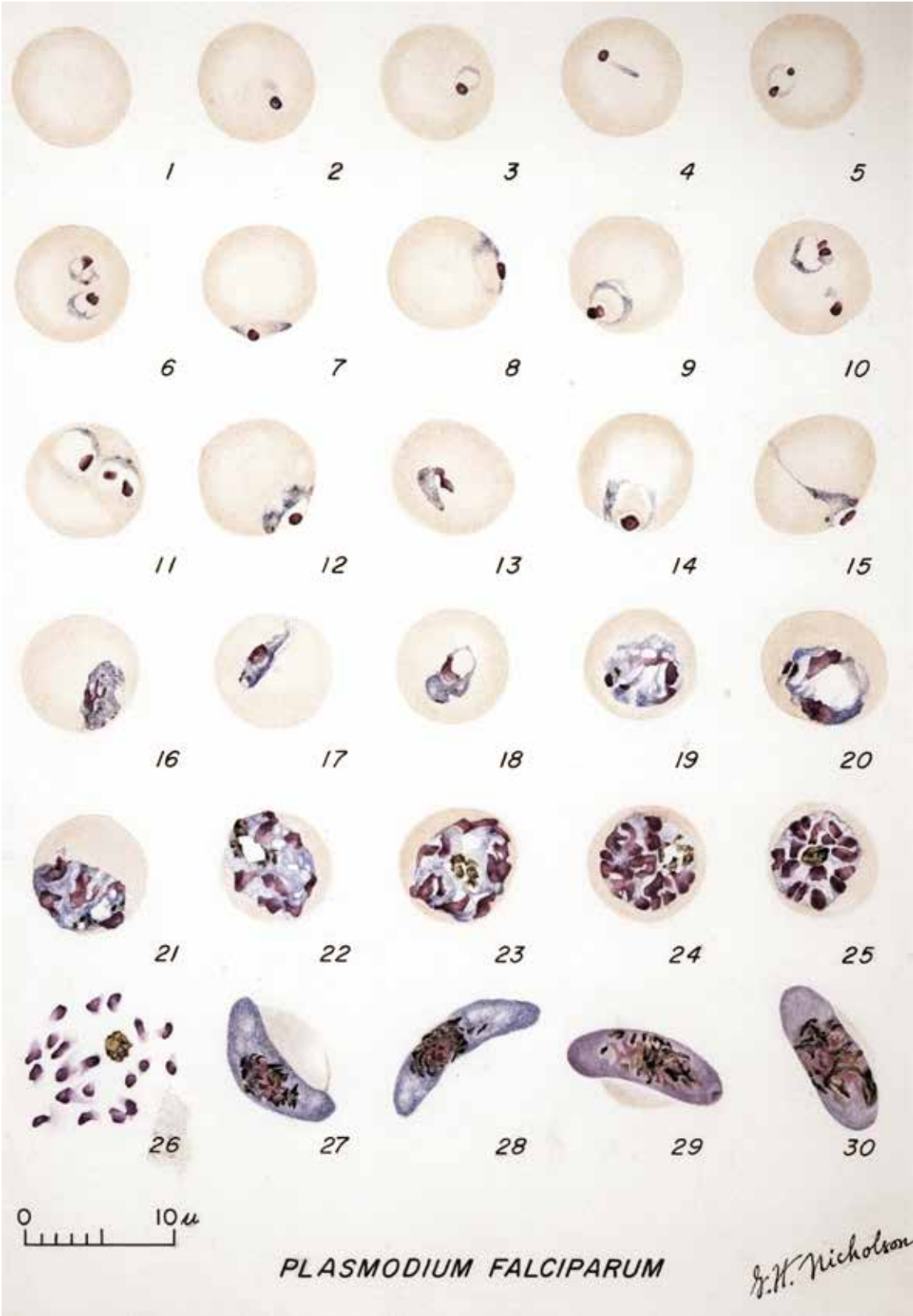


Figure 9.16.

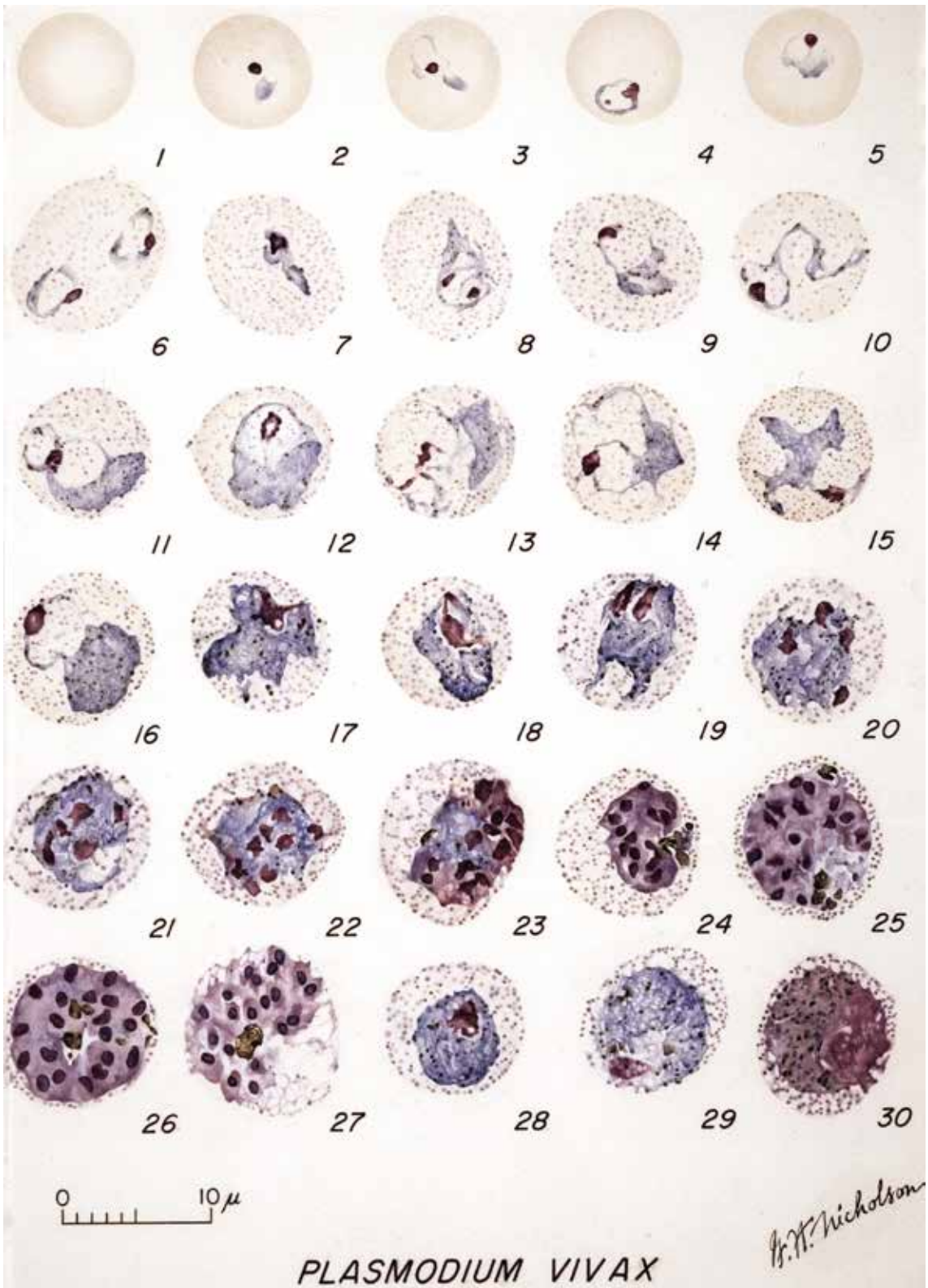


Figure 9.17.

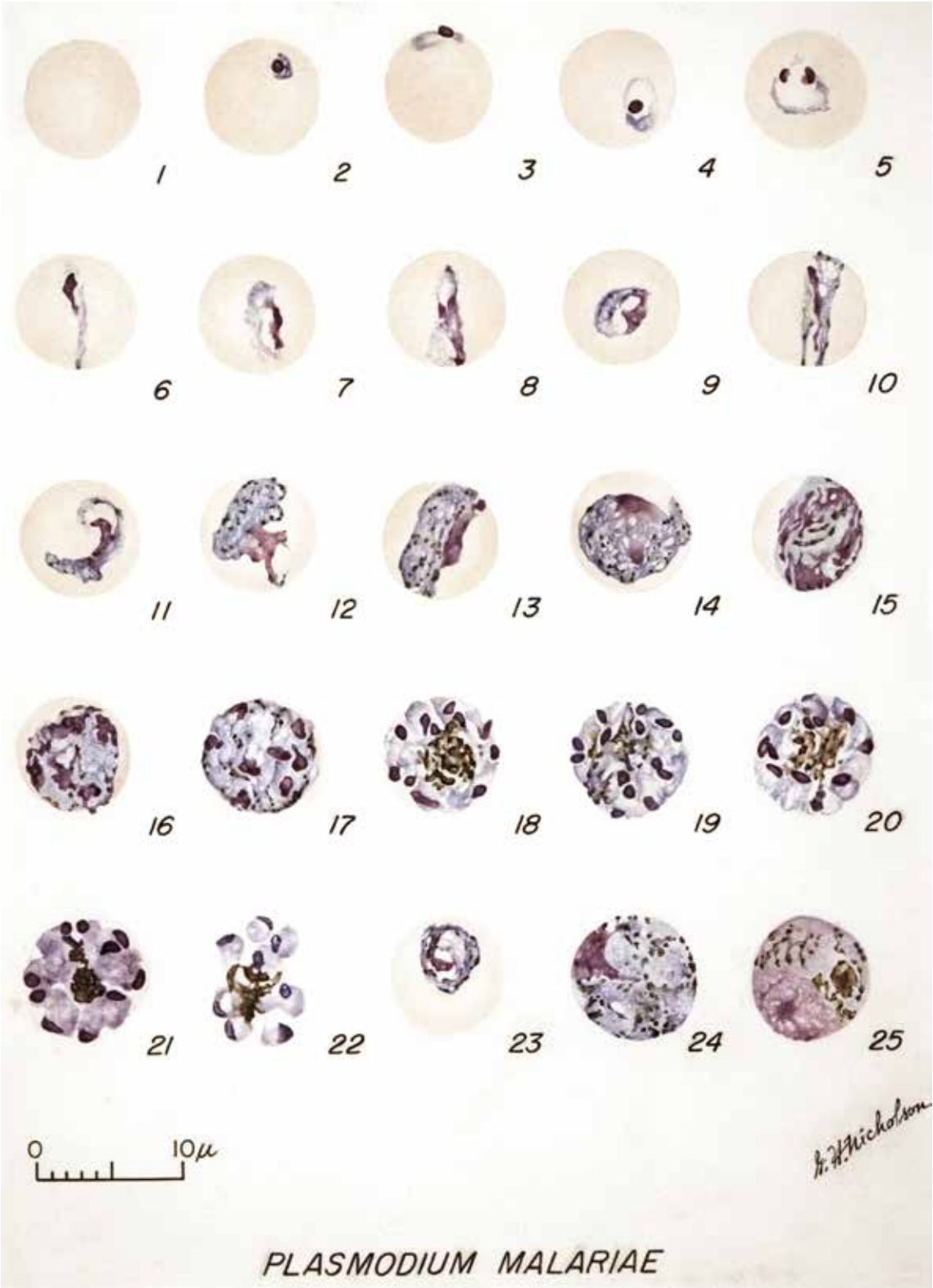


Figure 9.18.

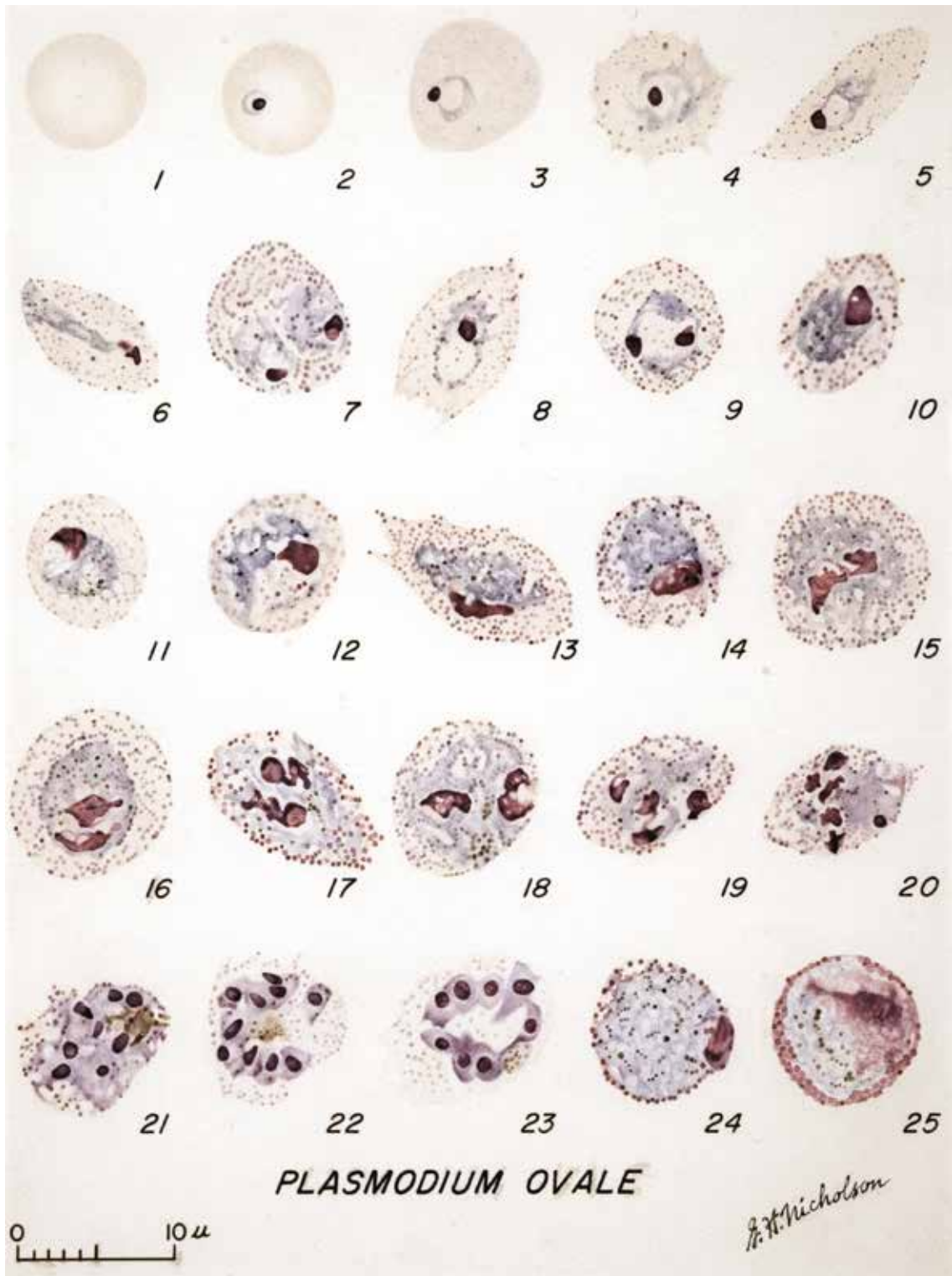


Figure 9.19.

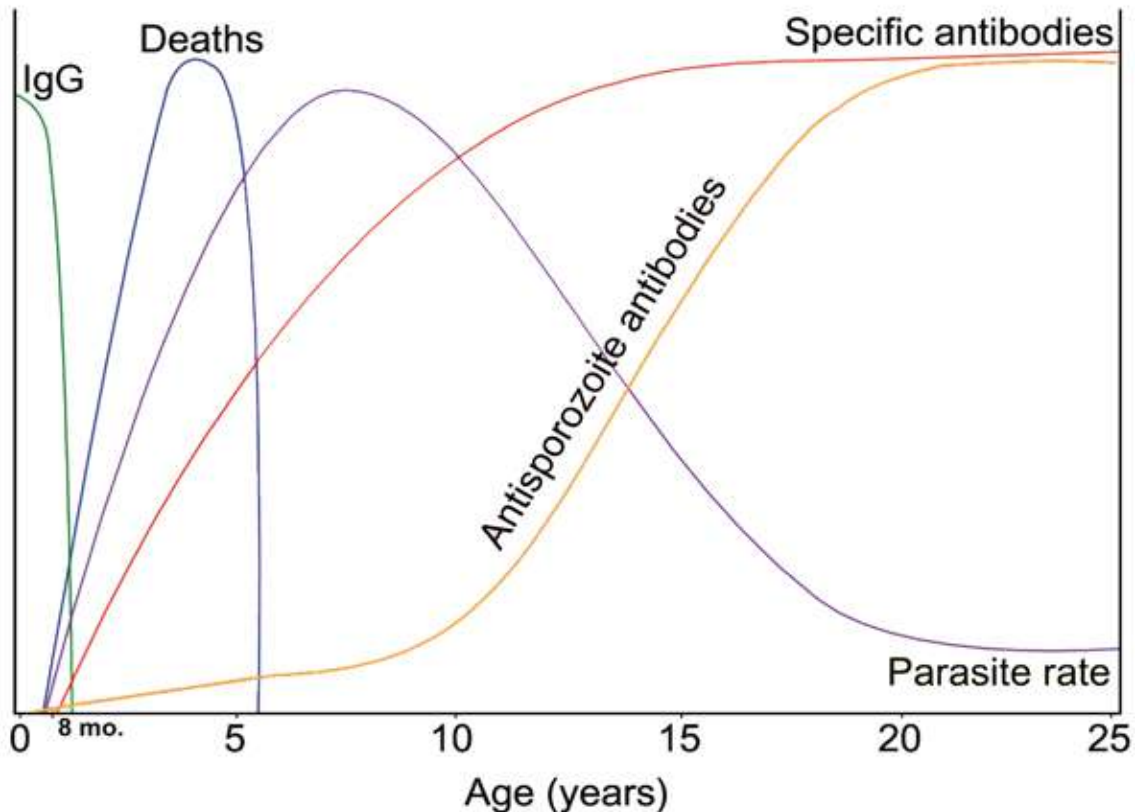


Figure 9.20. Graph indicating relationships between age of patient, susceptibility to infection, production of antibodies against different stages of parasite, and lethality of infection.

Once within the cell, the parasite grows, forming the ring-like early trophozoite and eventually enlarging to fill the cell. The organism then undergoes asexual division and becomes a schizont composed of merozoites. The parasites are nourished by the hemoglobin within the erythrocytes and produce a characteristic pigmented waste product called hemozoin. The erythrocytic cycle is completed when the red blood cell ruptures and releases merozoites that are then free to invade other erythrocytes.⁵³

Over some time, the asexual cycle becomes characteristically synchronous and periodic. *P. falciparum*, *P. vivax*, and *P. ovale* complete the development from invasion by merozoites to rupture of the erythrocyte within 48 hours, exhibiting tertian periodicity. *P. malariae*, which produces quartan malaria, requires

72 hours to complete the cycle. Counting the days is such that the first day is day one, and 48 hours later, on day three of the tertian day, fever is seen in *P. falciparum*, *P. vivax*, and *P. ovale*. When counting for *P. malariae*, day one is the first day, and 72 hours, or three days later, is the fourth day; the term quartan fever is then applied.¹⁸ The characteristic periodicity of the fever, based on synchronous infections, is not invariable; the early phases of infections are often not synchronous, and it takes time for the synchronized periodicity to develop. Immunologically naïve travelers usually become severely ill before developing the classic fever patterns, so the classic fever patterns are rarely seen in travelers.⁶⁷ Some infections may be due to two or more broods of parasites, with the periodicity of one independent of the others.

Infection with erythrocytic phase merozo-

ites can also occur when blood is transfused from an infected donor or via a contaminated needle shared among drug users. Malaria acquired in this manner is referred to as “induced” malaria.⁶⁸ No hypnozoites develop with induced malaria, even if the species is *P. vivax* and *P. ovale*, because the sporozoite stage, the only stage capable of infecting hepatocytes, is never present. Congenital malaria due to transplacental infection is perhaps more common than initially suspected due to its high rate of spontaneous clearance. Still, it appears to be increased in the context of HIV infection.⁶⁹⁻⁷³

Sexual Stages

Not all merozoites develop asexually. Some differentiate into the sexual forms – macrogametocytes (female) and microgametocytes (males) – which can complete their development only within the gut of an appropriate mosquito vector. On ingestion by the mosquito in the blood meal, the gametocytes shed their protective erythrocyte membrane in the gut. Male gametocytes initiate exflagellation (Fig. 9.11), a rapid process that produces up to eight active, sperm-like microgametes, each of which can eventually fertilize the macrogametes. The resulting zygotes elongate into diploid vermiform ookinetes (ō-ā-’kī- nēts\), which penetrate the gut wall and come to lie under the basement membrane (Fig. 9.12). The parasites then transform into oocysts (’ō-ā- sists\) within 24 hours of ingestion of the blood meal. Development of sporozoites follows, producing more than 1,000 of these now-haploid forms in each oocyst. They mature within 10–14 days, escape from the oocyst, and invade the salivary glands. When the mosquito bites another human host, a new cycle begins.

Although the different species have marked physiologic differences and some significant differences in the pathologic course they pursue, they are most simply differentiated

based on their morphology. The blood smear, typically fixed and stained with Giemsa or Wright solution, is the basis of the fundamental diagnostic test, although alternatives are now available. Commercially available malaria parasite detection and characterization methods are becoming increasingly sensitive, supplanting microscopy in more advanced laboratories (see Diagnosis).⁷⁴

Cellular and Molecular Pathogenesis

The release of cytokines following the rupture of infected erythrocytes induces fever and the consequent chills and sweating associated with malaria.⁷⁵ The pathogenesis of general malaise, myalgia, and headache appear to be related to the release of specific cytokines, and their levels correlate with disease severity.⁷⁶

Cerebral malaria is the most devastating manifestation of severe *P. falciparum* infection.⁷⁷ Cerebral malaria is caused by a complex process involving sequestration of erythrocytes, cytokine release, and endothelial activation.^{78, 79} The importance of various factors in the development of cerebral malaria has been a subject of debate for many years, with the development of the “school of sequestration” proponents as well as those more supportive of the “school of cytokines” providing a lively debate and contributing to an exciting number of publications advancing our understanding of this critical issue.^{80, 81} The mechanism of cytoadherence is thought to be related to the presence of histidine-rich “knobs” on the surface of the infected red blood cells that express strain-specific adhesive proteins (PfEMP1) and the subsequent attachment to appropriate receptors on the host endothelium (Figs. 9.13, 9.14).⁸² ⁸³ Although not essential for cytoadherence, the knobs seem to enhance binding.⁸⁴ The parasite induces these knobs and facilitates

endothelial cell binding by the infected erythrocytes to several endothelial targets.^{85, 86} Binding to endothelial cells involves several host cell receptors, including CD36, intercellular adhesion molecule 1 (ICAM1), and chondroitin sulfate A (CSA). In the brain, the intercellular adhesion molecule ICAM1 is likely the most important, while CD36 is likely the most important in other organs.⁵⁵ Cytoadherence in the placentae of women in their first pregnancies involves parasite binding to CSA.^{87, 88} Sequestration of malaria parasites in the placentae of primigravid females is a significant cause of death, fetal mortality, fetal wastage, and low birth weight.⁸⁸⁻⁹⁰

Malaria can also cause a significant degree of anemia, often in populations already suffering from anemia due to other causes. With *P. falciparum* malaria, anemia caused by hemolysis can be severe. Damage to the erythrocytes by intravascular hemolysis can exceed that caused by the rupture of the infected cells alone. Even uninfected cells have an increased osmotic fragility. Also present is bone marrow depression, which contributes to the anemia by decreasing the production of new erythrocytes.⁹¹ Disseminated intravascular coagulopathy occurs in severely infected individuals.⁹²

The spleen plays a significant role in host defense against malaria (Fig. 9.15). Parasitized cells accumulate in its capillaries and sinusoids, causing general congestion. The malarial pigment becomes concentrated in the spleen and is responsible for the darkening of this organ. Chronic infection, particularly with *P. malariae*, often causes persistent splenomegaly and is responsible for “big spleen disease,” or tropical splenomegaly syndrome, consisting of hepatomegaly, portal hypertension, anemia, leukopenia, and thrombocytopenia.⁹³ With *P. vivax* malaria, the spleen can become acutely enlarged and

is susceptible to rupture. A significant portion of the anemia seen in *P. vivax* malaria is driven by splenic clearance of non-infected erythrocytes.⁹⁴ The accumulated malarial pigment darkens the liver and shows degeneration and necrosis of the centrilobular regions. The gastrointestinal (GI) tract is also affected. There are focal hemorrhages, edema, and consequent malabsorption. The kidneys, particularly with severe *P. falciparum* malaria, show punctate hemorrhages and even tubular necrosis. Accumulation of hemoglobin in the tubules is responsible for hemoglobinuria, or blackwater fever, which occurs after repeated attacks of *falciparum* malaria and can be complicated by therapy with quinine.⁹⁵ Blackwater fever results from severe hemolysis exacerbated by the host’s immune response against the intracellular parasites.

Chronic infections with *P. malariae* can lead to nephrotic syndrome, characterized by focal hyalinization of the tufts of glomeruli and endothelial proliferation, apparently caused by the deposition of immune complexes.¹⁸ This process can lead to endocapillary cell proliferation and reduplication of the basement membrane.

Congenital malaria can develop with any of the species of *Plasmodia*, although the incidence of this complication is relatively low. The mechanism by which the fetus becomes infected is uncertain. Some investigators have postulated damage to the placenta as a prerequisite to congenital malaria. Still, it is also possible that the parasites can infect the fetus through an intact placenta or at the time of birth.⁷²

Malarial infections tend to suppress cell-mediated immune responses. It has been suggested that Burkitt’s lymphoma is caused by infection with the Epstein-Barr virus under the influence of immunosuppression by chronic *P. falciparum* malaria. Still, the

competing hypothesis is that it is the chronic immune activation that drives Burkitt's lymphoma.⁹⁶⁻⁹⁸

Pathogenesis of Malaria in the HIV-infected Population

The relationship between infection with malaria and HIV is a subject of great interest and intense scrutiny.⁹⁹ In Africa and other parts of the tropics, HIV and malaria overlap in their geographic ranges and the populations they infect.^{6, 100} The focus is often on how HIV infection predisposes individuals to increased malarial disease severity. There is evidence that malaria infection increases T-cell activation and causes both a decrease in CD4⁺ T cell counts and increases in viral loads.¹⁰¹⁻¹⁰³ There is also evidence to suggest a relationship between HIV-induced reduction of CD4⁺ T cell counts and a rise in malaria incidence.¹⁰³⁻¹⁰⁷ HIV does confer an increased susceptibility to symptomatic malaria.^{108, 109} There is conflicting information regarding the severity of clinical malaria when it manifests in HIV-infected individuals. Still, most evidence suggests that they experience higher levels of parasitemia and an increased risk for severe malaria.^{110, 111}

Clinical Disease

The most pronounced clinical manifestations of adult-onset malaria are periodic chills and fever, usually accompanied by frontal headache, fatigue, abdominal discomfort, and myalgia.^{55, 74} Fever may persist for several days before the typical periodicity develops. In contrast, young children often present with non-specific symptoms, including fever, cough, vomiting, and diarrhea. Symptoms of malaria usually first appear 10–15 days after the bite of the infected mosquito. However, delays of several months in the onset of symptoms and the appearance of parasites in peripheral blood are common, particularly for some strains of *P. vivax* found in temperate zones. Patients undergoing chemopro-

phylaxis may not develop symptoms until they stop taking the drug. The classic pattern of clinical disease consists of paroxysms of chills and fever, reaching 41 °C and lasting six hours, followed by sweating and defervescence.

Vomiting can also develop and may be intense. Difficulty breathing is seen in up to 25% of adults with severe malaria and approximately 40% of children with severe malaria.¹¹² Initially, there can be mild anemia with an elevation of the reticulocyte count. The leukocyte count tends to be normal or even low.¹¹³ The eosinophil count in acute malaria is characteristically low (eosinopenia). A robust rise in eosinophils and hemoglobin after completing malaria therapy is usually seen in successfully treated patients experiencing a good recovery.¹¹⁴

All forms of untreated malaria tend to become chronic, including those without a dormant hypnozoite stage. Repeated attacks are caused by recrudescence (recurrence of symptoms from persistent blood stages) or relapses (recurrence of symptoms and parasitemia from maturation of the hypnozoite stage). The development of host immunity can lead to a spontaneous cure of *P. falciparum* malaria within two years and of *P. vivax* and *P. ovale* malarias within five years. However, individuals are susceptible to reinfection during and after this period (Fig. 9.20). Infection with the quartan parasite, *P. malariae*, can potentially persist for the lifetime of the individual, with one report from China of 50 years between the time of acquisition and illness and another case of an individual with infection acquired in Greece more than 40 years and, possibly, as much as 70 years previously.^{18, 115} Untreated malaria, particularly *P. falciparum* malaria, can be fatal during the initial attack, an unfortunately frequent event in young children (Fig. 9.20).

Unexplained fever in patients who have received blood transfusions or are intravenous drug users may signal the presence of induced malaria. Induced malaria results from the direct transfer of infectious merozoites from the blood of an infected individual to a new host. Hypnozoites do not develop with a transfusion or induced malaria, even if the species is *P. vivax* and *P. ovale*, because the sporozoite stage, the only stage capable of infecting hepatocytes, is never present. An infant who develops a fever during the neonatal period should be suspected of malaria if the mother has been at risk of infection. Diagnostic tests for induced or congenital malaria are the same as for the conventional forms of the infection. It must be emphasized that neither induced nor congenital malaria has an exoerythrocytic cycle in the liver, and therapy directed against the liver cycle is not required.

P. vivax and *P. ovale* preferentially invade reticulocytes. Usually, only about 1–2% of red blood cells are parasitized with *P. vivax* and *P. ovale* infections. Clinical disease is typically mild. *P. malariae* tends to invade older erythrocytes, again limiting maximum parasitemia to about 1–2%. In contrast, *P. falciparum* attacks erythrocytes of all ages, permitting high levels of parasitemia.

Innate resistance to malaria is mediated by factors other than immune mechanisms. Several genetic factors in human populations confer varying levels of susceptibility to malaria.^{53, 116, 117} Individuals carrying the gene for sickle-cell hemoglobin receive some advantage against *P. falciparum* malaria. Those with sickle-cell trait (A and S hemoglobins) have a selective advantage over those with the hemoglobin AA genotype because the heterozygotes limit the severity of malaria. The hemoglobin SS individuals are also at an advantage, but their sickle-cell disease leads to early death.^{116–118} In areas of Africa with the highest frequency of this gene, it is estimated that the death rate

due to malaria required to fix this gene frequency may have exceeded 25%. Hemoglobin S is an excellent example of a balanced polymorphism.¹¹⁹

In 2011, a group of researchers demonstrated that sickle hemoglobin protects against malaria through induction of the expression of heme oxygenase-1, which prevents the accumulation of circulating heme.¹²⁰ This is particularly protective against cerebral malaria. Sickle hemoglobin can also inhibit the activation and expansion of CD8⁺ T cells, resulting in lower cellular activation and higher cellular reactivity in response to malarial antigens.^{120–122} Hemoglobin C mutations provide similar protection against *P. falciparum* malaria in certain ethnic groups.^{123, 124}

Glucose-6-phosphate dehydrogenase (G6PD) deficiency, β -thalassemia, and ovalocytosis (common in Southeast Asia) have been implicated as mediators of innate resistance against *P. falciparum* infection.¹²⁵ It has been suggested that thalassemia's protective effect may be related to enhanced immune recognition and clearance of parasitized erythrocytes.¹²⁶ Evidence also supports the idea that certain of these hemoglobinopathies reduce the intraerythrocytic multiplication of *Plasmodium* species.¹²⁷

Duffy blood type determinants are associated with receptor sites for *P. vivax* merozoites on the erythrocytes. While it is doubtful that the blood group carbohydrate is the actual receptor, most West Africans are negative for the Duffy blood type and have decreased susceptibility to infection with *P. vivax*.^{119, 128} Instead, they have a higher-than-normal incidence of infection with *P. ovale*.¹²⁹

Acquired immunity (incomplete nonsterile) develops after prolonged exposure to malaria and is characterized by low levels of parasitemia. Immune individuals have intermittent parasitemia with only mild symptoms.

This clinical state of tolerance and control of parasitemia to a lower level has been referred to as premunition, in contrast with classic immunity that leads to the clearance of a pathogen. Premunition does not last for life, and individuals returning to endemic areas after only one year away often have lost this protective immunity.¹³⁰⁻¹³²

Diagnosis (see Clinical Appendix)

For over a century, a definitive malaria diagnosis depended on the microscopic identification of the parasite on Giemsa-stained blood smears. This procedure permits not only the confirmation of the presence of the parasite but also makes possible the identification of the malaria species and an indication of the level of parasitemia in the infected host.¹³³ Under normal conditions, thick and thin smears should be examined. The clinician should take a thorough travel and exposure history when malaria is suspected. If the initial blood smears prove negative, new specimens should be drawn at 6-hour intervals.

Identifying malaria on thick and thin blood smears requires an experienced microscopist who is well-trained in parasite morphology. A British study indicated that at least 10% of positive slides are missed and not identified.¹³⁴ Antibody-based rapid diagnostic tests that are simple, specific, and do not require a highly trained microscopist have been introduced and are now widely available.¹³⁵ These rapid tests detect the presence or absence of PfHRP2, present in *P. falciparum*, and then allow the detection of other species and species-level discrimination using species-specific lactate dehydrogenase or aldolase antigens.¹³⁶⁻¹³⁸ Unfortunately, several rapid diagnostic tests can have poor sensitivity and specificity for diagnosing *P. knowlesi*.¹³⁹ Also concerning for the ongoing use of rapid tests is the report of PfHRP2 negative parasites.¹⁴⁰⁻¹⁴²

Several NAATs have been developed and employed in research and epidemiological studies.¹⁴³ Introducing portable platforms (e.g., loop-mediated isothermal amplification (LAMP) assays) may allow molecular testing to move from the research setting to field diagnosis.¹⁴⁴⁻¹⁴⁶

Identifying parasite genes conferring resistance to antimalarial drugs has permitted the development of mutation-specific PCR primers that can readily identify resistant parasites. Such detection systems are available for pyrimethamine, sulfadoxine, cycloguanil, chloroquine, and artemisinin.¹⁴⁷⁻¹⁵¹ Epidemiologic studies requiring information on sporozoite inoculation rates by mosquito vectors have been facilitated by ELISA systems using species-specific monoclonal antibodies directed against the predominant surface antigens of the sporozoites.¹⁵² The capacity for rapidly determining the proportion of mosquito populations coming to feed that have infectious sporozoites in their salivary glands allows accurate prediction of the risk of infection or assessment of the effects of intervention strategies for controlling malaria.

Treatment (see Clinical Appendix)

Due to the worldwide spread of drug resistance, the long reliance on chloroquine to treat *P. falciparum* is no longer tenable.¹⁵³ The two primary choices for treating severe malaria are the cinchona alkaloids (quinine and quinidine) and the artemisinin derivatives (artesunate, artemether, and artemotil). Parenteral artesunate is the most rapidly acting of the artemisinin compounds and is associated with more rapid clearance of parasitemia than treatment with the cinchona alkaloid quinine.^{154, 155} Artesunate treatment has demonstrated a significant reduction in mortality in adults and children compared to treatment with quinine.^{154, 155} While arte-

misinin-based therapy was initially recommended as primary therapy in areas of high drug resistance, it is now first-line therapy in most cases of severe malaria.^{156, 157}

A significant and striking exception to this worldwide recommendation has been malaria treatment in the United States. For parts of the world where artesunate is unavailable, quinine and quinidine are alternative therapies. In 1991, parenteral quinine was no longer stocked by the Centers for Disease Control (CDC) when it was decided that parenteral quinidine gluconate could serve as an alternative.¹⁵⁸ Quinidine gluconate remained the only approved therapy by the FDA for the parenteral treatment of severe malaria until 2019, when it was announced that intravenous artesunate would become available in the United States to treat severe malaria. Intravenous artesunate is now commercially available in the United States. Due to the high cost and low frequency with which severe malaria is encountered, many hospitals need to keep this life-saving medication in stock.¹⁵⁹ Given the favorable comparative data, intravenous artesunate should be considered early in therapy.¹⁶⁰ In addition to artesunate, quinine, or quinidine, a second long-acting medication is required, such as doxycycline, tetracycline, or clindamycin, for successful treatment.

When selecting treatment, there are several essential factors that the treating clinician must consider, including the severity of the clinical manifestation (uncomplicated versus severe), the specific malaria being treated, and drug susceptibility or resistance. When treating severe malaria infections with quinidine, care must include monitoring patients with standard cardiac monitors, careful blood glucose tracking, cautious fluid management, and delayed initiation of enteral feeding, factors critical to successful treatment outcomes.³⁵ Seizures may need to be

managed with anti-seizure medications or benzodiazepines, and airway protection may be required in severe cases. Broad-spectrum intravenous antibiotics may be indicated in some instances due to the high rate of bacterial sepsis that can occur in severe malaria.¹⁶¹

Exchange transfusions, once felt to be essential in the management of severe malaria, are no longer recommended by the CDC since a meta-analysis of previous studies revealed no significant survival advantage.¹⁶² Blood transfusions for patients have been recommended based on the results of small randomized trials and a few large observational trials. Transfusion has been recommended if hemoglobin levels are below four g/dL in all cases or if hemoglobin levels are below six g/dL and there is parasitemia greater than 20% or other concerning clinical features.^{163, 164}

In contrast to severe or complicated malaria, non-complicated malaria is defined as symptomatic malaria without any severe malaria criteria. The patient demonstrates lower levels of parasitemia, retains the ability to take oral medicine, and displays no evidence of vital organ dysfunction. Chloroquine can treat uncomplicated malaria if a patient has a *P. falciparum* infection acquired from a region without chloroquine resistance, such as the Caribbean and Central America west of the Panama Canal.¹⁶⁵ In the rest of the world, resistance to chloroquine is so frequently reported that every patient must be assumed to have a resistant form of *P. falciparum*. If the origin of the infection is unknown, as may be the case with induced malaria, one must treat the infection as if the organisms are resistant. Currently, artemisinin combination therapy is the recommended first-line therapy for all human malaria.¹⁶⁶ There are several available forms of artemisinin combination therapy, with no one choice superior to the other.¹⁶⁶ Artemisinin resistance is reported in several regions and is associated

with a slower clearance of parasitemia.^{167, 168}

Alternative drugs with knowledge of local resistance rates may be selected on a region-by-region basis.¹⁶⁹ Fansidar, a pyrimethamine-sulfadoxine combination, lost effectiveness as a replacement for chloroquine in East African countries but still may have a role as part of intermittent preventive therapy in pregnancy.¹⁷⁰⁻¹⁷² Mefloquine can be given as part of combination therapy with a second agent, such as doxycycline, with cure rates estimated to be greater than 90% in areas without resistance.¹⁷³ Malarone (atovaquone-proguanil) is an effective option that has demonstrated superior efficacy over mefloquine treatment and may have a better safety profile.^{174, 175} The Sentinel sites in the International Centers of Excellence for Malaria Research provide essential data to monitor the emergence of drug resistance globally and the underlying mechanisms to further understand the impact.¹⁷⁶

When the infecting strain is *P. vivax* or *P. ovale*, treatment may not resolve infection by the hypnozoite liver stage. To prevent relapses, these must be cleared with a course of primaquine. Primaquine need not be started immediately but can be deferred until the patient has recovered from the acute attack. Primaquine tends to cause hemolysis in individuals with G6PD deficiency. The drug should not be administered until the proper test for this enzyme has been performed. Tafenoquine is an alternative for preventing relapses.¹⁷⁷⁻¹⁷⁹

Chemoprophylaxis for Malaria (see Clinical Appendix)

In areas without chloroquine resistance, such as the Caribbean and Central America west of the Panama Canal, chloroquine can be used for chemoprophylaxis, as there is little resistance to the drug in this region.¹⁶⁵ Mefloquine is an inexpensive option with weekly dosing that can be used in most of the world,

except for localized areas where resistance has been reported. Many hesitate to prescribe or take mefloquine due to concerns regarding its potential to induce psychosis.¹⁸⁰ Malarone (atovaquone-proguanil) is a prophylactic drug for travelers to areas where *P. falciparum* is resistant to other medications but requires once-daily dosing and is often very expensive. Daily doxycycline is another alternative, but patients should be cautioned about the associated photosensitivity. In some regions of the world, primaquine is used as a form of chemoprophylaxis, but the efficacy of this approach may be inferior to other chemoprophylaxis regimens. G6PD testing should be performed before using primaquine due to the danger of hemolysis.¹⁸¹

Prevention and Control

More than 300 species of *Anopheles* mosquitoes exist, but only about 60 are considered important malaria vectors. Some factors that influence the efficiency of the insect are their feeding habits (most importantly, a preference for human blood), longevity, susceptibility to infection with the malarial parasite, and the size of the mosquito population (seasonal variability).

The variability of the parasite plays an essential role in the pathogenicity of the disease. For example, geographic strains of *P. vivax* show markedly different incubation periods and patterns of relapses, and *P. falciparum* shows considerable variability in its responses to anti-malarial drugs. The susceptibility of geographic strains of vector mosquitoes may be highly variable.

Most malaria in the United States, since the 1960s, has been imported. The wars in Korea and Vietnam increased the numbers of these imported cases because of the returning infected service personnel. Refugees or immigrants from endemic areas constitute

the most significant number of imported cases. In addition, there is a steady incidence of malaria among travelers returning from endemic regions. Autochthonous (\au·toch·tho·nous\) infections are rare in the United States, despite large, persistent populations of the anopheline vectors, *An. quadrimaculatus* in the East and *An. freeborni* in the West.¹¹ Outbreaks of *P. vivax* in southern California have been associated with the vector species *An. hermsi*.¹⁸² There are rare reports of malaria transmission in the United States, usually limited to one or two cases transmitted by local anopheline mosquitoes.¹¹

In addition to chemoprophylaxis, travelers should avoid or minimize contact with mosquitoes. Since most anophelines bite at night, sleeping under insecticide-treated bed netting and, if possible, in rooms fitted with window screens is effective.¹⁸³ Clothing that covers much of the skin and insect repellents, particularly those containing DEET or picaridin, are valuable adjuncts to transmission prevention.

Controlling the mosquito vector remains the most practical method for wide-scale malaria control. A reduction in the number of mosquitoes through drainage or modification of breeding sites has been accomplished in some areas. Insecticides may offer the best but increasingly less acceptable method for reducing populations of mosquitoes or interrupting transmission by targeting only those infected mosquitoes coming to feed in houses. The rising costs of these insecticides and the development of insect resistance have severely limited their application and usefulness.¹⁸⁴ Insecticide-impregnated bed nets have been shown to significantly impact the morbidity and mortality of infection due to *P. falciparum* and *P. vivax* in China and *P. falciparum* in Africa.^{185, 186} Malaria control schemes based on genetic modification of the capacity of vector mosquitoes to trans-

mit the parasite have been suggested.¹⁸⁷ In addition, developing more efficient methods for introducing advantageous genes into the mosquito genome and strategies for replacing vector populations in the field with mosquitoes unable to transmit the parasite are being investigated.^{188, 189}

A malaria vaccine remains the “holy grail” of control strategies. For over 50 years, researchers have been attempting to find antigens that could induce protective immunity. After years of sporadic advances, vaccine research was reinvigorated by the demonstration that animals could develop immunity to infection with sporozoites and stimulated by Trager and Jensen’s development of methods for the in vitro cultivation of the asexual and sexual stages of *P. falciparum*.¹⁹⁰ The revolution of molecular biology made the identification of specific genes coding for particular antigens and sub-unit vaccines possible.¹⁹¹

The vaccine investigators have targeted three phases of the malaria life cycle.^{192, 193} Vaccines directed against the pre-erythrocytic stages of the parasite are intended to prevent infection by blocking the invasion or development of sporozoites freshly injected by a feeding mosquito or the development of the parasite in the liver. Secondly, it has been suggested that even partial efficacy (the blockage of most pre-erythrocytic development) could reduce the intensity of the primary infection and be helpful in concert with antigens directed against other stages. Because such vaccines may have short-term efficacy, the target population for pre-erythrocytic stage vaccines has usually been considered to be non-immune individuals moving through malaria-endemic areas, including tourists and military personnel. Even with a short life, such vaccines could be helpful in areas of low transmission or children and women of childbearing age in areas of high transmission.

Vaccines directed against the parasite's erythrocytic (blood) stages are not expected to induce sterilizing immunity or prevent infection. Instead, a successful vaccine against the parasite's erythrocytic (blood) stages could reduce the parasite burden, reducing deaths and morbidity. The primary target for blood-stage vaccines are children and pregnant women in areas of intense transmission.

Vaccines directed against the mosquito (sexual) stages of the parasite are designed to block the development of the parasite in the mosquito vector. An effective vaccine, a so-called altruism vaccine, could interrupt transmission to additional victims. Combined with other antigens, a transmission-blocking component could prevent the spread of parasites

resistant to other vaccines. A transmission-blocking vaccine could be used in an eradication scheme or to prevent epidemics in areas of unstable malaria transmission.

Two malaria vaccines, RTS,S (Mosquirix) and R21/Matrix-M, have proven efficacy and are available in sub-Saharan Africa.^{194, 195} Both vaccines target the circumsporozoite protein and require multiple doses.^{196, 197} In 2024, Cameroon became the first African nation to launch a malaria vaccine campaign with RTS, S.

The investigational malaria vaccines are listed by stage of clinical development on The Malaria Vaccine Initiative website (www.malariavaccine.org).

References

1. Moyes, C. L., et al., Defining the geographical range of the *Plasmodium knowlesi* reservoir. *PLoS neglected tropical diseases* **2014**, *8* (3), e2780.
2. CDC, Progress toward interruption of wild poliovirus transmission-worldwide, January -March. *MMWR* **2005**, *54*, 408-12.
3. Greenwood, B. M., et al., Malaria. *Lancet* **2005**, *365* (9469), 1487-98.
4. Carter, R.; Mendis, K. N., Evolutionary and historical aspects of the burden of malaria. *Clinical microbiology reviews* **2002**, *15* (4), 564-94.
5. WHO, World Malaria Report 2015. *Geneva, World Health Organization* **2015**.
6. WHO World malaria report 2017. (accessed March 3, 2018).
7. The, L., Malaria in 2022: a year of opportunity. *Lancet* **2022**, *399* (10335), 1573.
8. Newby, G., et al., The path to eradication: a progress report on the malaria-eliminating countries. *Lancet* **2016**, *387* (10029), 1775-84.
9. Williams, L. L., Jr., Malaria eradication in the United States. *Am J Public Health Nations Health* **1963**, *53*, 17-21.
10. Andrews, J. M.; Gilbertson, W. E., Blueprint for malaria eradication in the United States. *J Natl Malar Soc* **1948**, *7* (3), 167-70.
11. Blackburn, D., et al., Outbreak of Locally Acquired Mosquito-Transmitted (Autochthonous) Malaria - Florida and Texas, May-July 2023. *MMWR Morb Mortal Wkly Rep* **2023**, *72* (36), 973-978.
12. Voloc, A.; Djoba Siawaya, J. F., Malaria beyond its predominant endemic regions: Emerging threat or sporadic events? *Front Med (Lausanne)* **2022**, *9*, 969271.
13. Cholera, R., et al., Impaired cytoadherence of *Plasmodium falciparum*-infected erythrocytes containing sickle hemoglobin. *Proc Natl Acad Sci U S A* **2008**, *105* (3), 991-6.
14. Taylor, S. M., et al., Hemoglobinopathies and the clinical epidemiology of malaria: a systematic review and meta-analysis. *Lancet Infect Dis* **2012**, *12* (6), 457-68.
15. Cyrklaff, M., et al., Hemoglobins S and C interfere with actin remodeling in *Plasmodium falciparum*-infected erythrocytes. *Science* **2011**, *334* (6060), 1283-6.
16. Adams, H. C., The Genuine Works of Hippocrates. **1886**, The Epidemics Book 1.
17. Laveran, A., Note sur un nouveau parasite trouve' dans le sang de plusieurs malades atteints de fie`vre palustre. *Bull. Acad. Med* **1880**, *8*, 1235-1236.

18. Collins, W. E.; Jeffery, G. M., *Plasmodium malariae*: parasite and disease. *Clin Microbiol Rev* **2007**, *20* (4), 579-92.
19. Kean, B. H., et al., Tropical medicine and parasitology: classic investigations. *Cornell University Press, Ithaca, NY* **1978**.
20. Harrison, G., Mosquitoes, Malaria, and Man: a history of the hostilities since 1880. 1978.
21. Bruce-Chwat, L. J., et al., History of malaria from pre-history to eradication. In *Malaria Principles and Practice of Malariology* eds Churchill Livingstone Edinburgh pp **1988**, 1-59.
22. Shortt, H. E.; Garnham, P. C., Pre-erythrocytic stage in mammalian malaria parasites. *Nature* **1948**, *161* (4082), 126.
23. Krotoski, W. A., et al., Relapses in primate malaria: discovery of two populations of exoerythrocytic stages. Preliminary note. *Br Med J* **1980**, *280* (6208), 153-4.
24. Krotoski, W. A., et al., Demonstration of hypnozoites in sporozoite-transmitted *Plasmodium vivax* infection. *Am J Trop Med Hyg* **1982**, *31* (6), 1291-3.
25. Markus, M. B., Possible support for the sporozoite hypothesis of relapse and latency in malaria. *Trans R Soc Trop Med Hyg* **1976**, *70* (5-6), 535.
26. Markus, M. B., The malarial hypnozoite. *Lancet* **1980**, *1* (8174), 936.
27. Haggis, A. W., Fundamental errors in the early history of *Cinchona*. *Bull Hist Med* **1941**, *10*, 567-568.
28. Meshnick, S. R.; Sherman, I. W., From quinine to Qinghaosu: Historical perspectives. 1998; p 341-353.
29. Honigsbaum, M., The Fever Trail: In search of the cure for malaria. *Nature Medicine* **2001**.
30. Rocco, F., Quinine: Malaria and the quest for a cure that changed the world. **2003**.
31. Permin, H., et al., On the history of *Cinchona* bark in the treatment of Malaria. *Dan Medicinhist Arbog* **2016**, *44*, 9-30.
32. Phyto, A. P., et al., Clinical impact of vivax malaria: A collection review. *PLoS Med* **2022**, *19* (1), e1003890.
33. Fuehrer, H. P.; Noedl, H., Recent advances in detection of *Plasmodium ovale*: implications of separation into the two species *Plasmodium ovale wallikeri* and *Plasmodium ovale curtisi*. *Journal of clinical microbiology* **2014**, *52* (2), 387-91.
34. Nolder, D., et al., An observational study of malaria in British travellers: *Plasmodium ovale wallikeri* and *Plasmodium ovale curtisi* differ significantly in the duration of latency. *BMJ Open* **2013**, *3* (5).
35. Vinetz, J. M., et al., *Plasmodium malariae* infection in an asymptomatic 74-year-old Greek woman with splenomegaly. *The New England journal of medicine* **1998**, *338* (6), 367-71.
36. Garnham, P. C., Swellengrebel lecture. Hypnozoites and 'relapses' in *Plasmodium vivax* and in vivax-like malaria. *Tropical and geographical medicine* **1988**, *40* (3), 187-95.
37. R., K.; B.M., D. G., A study of monkey-malaria, and its experimental transmission to man. *Ind Med Gaz* **1932**, *67*, 301-21.
38. Kantele, A.; Jokiranta, T. S., Review of cases with the emerging fifth human malaria parasite, *Plasmodium knowlesi*. *Clin Infect Dis* **2011**, *52* (11), 1356-62.
39. White, N. J., *Plasmodium knowlesi*: the fifth human malaria parasite. *Clin Infect Dis* **2008**, *46* (2), 172-3.
40. Iwagami, M., et al., First case of human infection with *Plasmodium knowlesi* in Laos. *PLoS Negl Trop Dis* **2018**, *12* (3), e0006244.
41. White, N. J., Sharing malarias. *Lancet (London, England)* **2004**, *363* (9414), 1006.
42. Abegunde, A. T., Monkey malaria in man. *Lancet (London, England)* **2004**, *364* (9441), 1217.
43. Singh, B., et al., A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. *Lancet (London, England)* **2004**, *363* (9414), 1017-24.
44. Brasil, P., et al., Outbreak of human malaria caused by *Plasmodium simium* in the Atlantic Forest in Rio de Janeiro: a molecular epidemiological investigation. *Lancet Glob Health* **2017**, *5* (10), e1038-e1046.
45. Coatney, G. R., The simian malarias: zoonoses, anthroponoses, or both? *The American journal of tropical medicine and hygiene* **1971**, *20* (6), 795-803.
46. Ta, T. H., et al., First case of a naturally acquired human infection with *Plasmodium cynomolgi*.

- Malar J* **2014**, *13*, 68.
47. Vanderberg, J. P., *Plasmodium berghei*: quantitation of sporozoites injected by mosquitoes feeding on a rodent host. *Exp Parasitol* **1977**, *42* (1), 169-81.
 48. Yamauchi, L. M., et al., Plasmodium sporozoites trickle out of the injection site. *Cell Microbiol* **2007**, *9* (5), 1215-22.
 49. Sidjanski, S.; Vanderberg, J. P., Delayed migration of *Plasmodium* sporozoites from the mosquito bite site to the blood. *Am J Trop Med Hyg* **1997**, *57* (4), 426-9.
 50. Sinnis, P.; Coppi, A., A long and winding road: the Plasmodium sporozoite's journey in the mammalian host. *Parasitol Int* **2007**, *56* (3), 171-8.
 51. Meis, J. F.; Verhave, J. P., Exoerythrocytic development of malarial parasites. *Advances in parasitology* **1988**, *27*, 1-61.
 52. Hollingdale, M. R., Biology and immunology of sporozoite invasion of liver cells and exoerythrocytic development of malaria parasites. *Progress in allergy* **1988**, *41*, 15-48.
 53. Sherman, I. W., Malaria: Parasite Biology, Pathogenesis and Protection. ASM Press, Washington, D.C. : **1998**.
 54. Cha, S. J., et al., CD68 acts as a major gateway for malaria sporozoite liver infection. *J Exp Med* **2015**, *212* (9), 1391-403.
 55. White, N. J., et al., Malaria. *Lancet* **2014**, *383* (9918), 723-35.
 56. Coatney, G. R., Relapse in malaria--an enigma. *The Journal of parasitology* **1976**, *62* (1), 3-9.
 57. Schmidt, L. H., Compatibility of relapse patterns of *Plasmodium cynomolgi* infections in rhesus monkeys with continuous cyclical development and hypnozoite concepts of relapse. *The American journal of tropical medicine and hygiene* **1986**, *35* (6), 1077-99.
 58. Krotoski, W. A., Discovery of the hypnozoite and a new theory of malarial relapse. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1985**, *79* (1), 1-11.
 59. Adekunle, A. I., et al., Modeling the dynamics of *Plasmodium vivax* infection and hypnozoite reactivation *in vivo*. *PLoS Negl Trop Dis* **2015**, *9* (3), e0003595.
 60. Hulden, L.; Hulden, L., Activation of the hypnozoite: a part of *Plasmodium vivax* life cycle and survival. *Malar J* **2011**, *10*, 90.
 61. Krotoski, W. A., The hypnozoite and malarial relapse. *Prog Clin Parasitol* **1989**, *1*, 1-19.
 62. Galappaththy, G. N., et al., Primaquine for preventing relapse in people with *Plasmodium vivax* malaria treated with chloroquine. *Cochrane Database Syst Rev* **2013**, *10*, CD004389.
 63. Dvorak, J. A., et al., Invasion of erythrocytes by malaria merozoites. *Science (New York, N.Y.)* **1975**, *187* (4178), 748-50.
 64. Egan, E. S., et al., Malaria. A forward genetic screen identifies erythrocyte CD55 as essential for *Plasmodium falciparum* invasion. *Science* **2015**, *348* (6235), 711-4.
 65. Gruszczyk, J., et al., Transferrin receptor 1 is a reticulocyte-specific receptor for *Plasmodium vivax*. *Science* **2018**, *359* (6371), 48-55.
 66. Aikawa, M., et al., Erythrocyte entry by malarial parasites. A moving junction between erythrocyte and parasite. *The Journal of cell biology* **1978**, *77* (1), 72-82.
 67. Kotlyar, S.; Rice, B. T., Fever in the returning traveler. *Emerg Med Clin North Am* **2013**, *31* (4), 927-44.
 68. Garvey, G., et al., Transfusion-induced malaria after open-heart surgery. *New York state journal of medicine* **1975**, *75* (4), 602-3.
 69. Covell, G., Congenital malaria. *Trop Dis Bull* **1950**, *47*, 1147-1167.
 70. Mwangoka, G. W., et al., Congenital *Plasmodium falciparum* infection in neonates in Muheza District, Tanzania. *Malar J* **2008**, *7*, 117.
 71. Ogolla, S., et al., Reduced Transplacental Transfer of a Subset of Epstein-Barr Virus-Specific Antibodies to Neonates of Mothers Infected with *Plasmodium falciparum* Malaria during Pregnancy. *Clin Vaccine Immunol* **2015**, *22* (11), 1197-205.
 72. Ouedraogo, A., et al., Transplacental Transmission of *Plasmodium falciparum* in a Highly Malaria Endemic Area of Burkina Faso. *J Trop Med* **2012**, *2012*, 109705.
 73. Falade, C., et al., Epidemiology of congenital malaria in Nigeria: a multi-centre study. *Trop Med Int Health* **2007**, *12* (11), 1279-87.

74. Hanscheid, T., Diagnosis of malaria: A review of alternatives to conventional microscopy. *Clin Lab Haem* **1999**, *21*, 235-245.
75. Gallego-Delgado, J., et al., A surprising role for uric acid: the inflammatory malaria response. *Curr Rheumatol Rep* **2014**, *16* (2), 401.
76. Prakash, D., et al., Clusters of cytokines determine malaria severity in *Plasmodium falciparum*-infected patients from endemic areas of Central India. *J Infect Dis* **2006**, *194* (2), 198-207.
77. WHO, Severe and complicated malaria. *Trans R Soc Trop Med Hyg* **1986**, 1-50.
78. Wahlgren, M., et al., Malaria: Chapter 10. Cytoadherence and rosetting in the pathogenesis of severe malaria. CRC Press: **1999**; p 289-327.
79. Frevert, U.; Nacer, A., Fatal cerebral malaria: a venous efflux problem. *Front Cell Infect Microbiol* **2014**, *4*, 155.
80. Storm, J.; Craig, A. G., Pathogenesis of cerebral malaria--inflammation and cytoadherence. *Front Cell Infect Microbiol* **2014**, *4*, 100.
81. Grau, G. E.; Craig, A. G., Cerebral malaria pathogenesis: revisiting parasite and host contributions. *Future Microbiol* **2012**, *7* (2), 291-302.
82. Cooke, B., et al., *Falciparum* malaria: sticking up, standing out and out-standing. *Parasitol Today* **2000**, *16* (10), 416-20.
83. Kilejian, A., Characterization of a protein correlated with the production of knob-like protrusions on membranes of erythrocytes infected with *Plasmodium falciparum*. *Proc Natl Acad Sci U S A* **1979**, *76* (9), 4650-3.
84. Ruangjirachuporn, W., et al., Cytoadherence of knobby and knobless *Plasmodium falciparum*-infected erythrocytes. *Parasitology* **1991**, *102 Pt 3*, 325-34.
85. Udeinya, I. J., et al., *Falciparum* malaria-infected erythrocytes specifically bind to cultured human endothelial cells. *Science (New York, N.Y.)* **1981**, *213* (4507), 555-7.
86. Chisti, A. H., et al., Isolation of skeleton-associated knobs from human blood cells infected with the malaria parasite *Plasmodium falciparum*. *Mol Biochem Parasit* **1992**, *52*, 293-297.
87. Miller, L. H., et al., Malaria pathogenesis. *Science (New York, N.Y.)* **1994**, *264* (5167), 1878-83.
88. Duffy, P. E.; Fried, M., Antibodies that inhibit *Plasmodium falciparum* adhesion to chondroitin sulfate A are associated with increased birth weight and the gestational age of newborns. *Infection and immunity* **2003**, *71* (11), 6620-3.
89. Miller, L. H.; Smith, J. D., Motherhood and malaria. *Nature medicine* **1998**, *4* (11), 1244-5.
90. Duffy, P. E.; Fried, M., *Plasmodium falciparum* adhesion in the placenta. *Current opinion in microbiology* **2003**, *6* (4), 371-6.
91. Dormer, P., et al., Ineffective erythropoiesis in acute human *P. falciparum* malaria. *Blut* **1983**, *46* (5), 279-88.
92. Miller, L. H., et al., Malaria pathogenesis. *Science* **1994**, *264* (5167), 1878-83.
93. Looareesuwan, S., et al., Dynamic alteration in splenic function during acute *falciparum* malaria. *The New England journal of medicine* **1987**, *317* (11), 675-9.
94. Douglas, N. M., et al., The anaemia of *Plasmodium vivax* malaria. *Malar J* **2012**, *11*, 135.
95. Bruneel, F., et al., [Blackwater fever]. *Presse medicale (Paris, France : 1983)* **2002**, *31* (28), 1329-34.
96. Ernberg, I., et al., Malaria: Molecular and Clinical Aspects: Burkitt's lymphoma and malaria. Harwood Academic Publishers, Amsterdam: **1999**; p 370-399.
97. O'Byrne, K. J.; Dalglish, A. G., Chronic immune activation and inflammation as the cause of malignancy. *Br J Cancer* **2001**, *85* (4), 473-83.
98. Moormann, A. M., et al., The company malaria keeps: how co-infection with Epstein-Barr virus leads to endemic Burkitt lymphoma. *Curr Opin Infect Dis* **2011**, *24* (5), 435-41.
99. Murray, C. J., et al., Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **2014**, *384* (9947), 1005-70.
100. Collaborators, G. H., Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980-2015: the Global Burden of Disease Study 2015. *Lancet HIV* **2016**, *3* (8), e361-e387.
101. Froebel, K., et al., Activation by malaria antigens renders mononuclear cells susceptible to HIV

- infection and re-activates replication of endogenous HIV in cells from HIV-infected adults. *Parasite Immunol* **2004**, 26 (5), 213-7.
102. Mermin, J., et al., Association between malaria and CD4 cell count decline among persons with HIV. *J Acquir Immune Defic Syndr* **2006**, 41 (1), 129-30.
 103. Kublin, J. G., et al., Effect of *Plasmodium falciparum* malaria on concentration of HIV-1-RNA in the blood of adults in rural Malawi: a prospective cohort study. *Lancet (London, England)* **2005**, 365 (9455), 233-40.
 104. Grimwade K; French, N., HIV infection as a cofactor for severe *falciparum* malaria in adults living in a region of unstable malaria transmission in South Africa. *Aids* **2004**, 18, 547-554.
 105. Mount, A. M., et al., Impairment of humoral immunity to *Plasmodium falciparum* malaria in pregnancy by HIV infection. *Lancet* **2004**, 363 (9424), 1860-7.
 106. Whitworth, J. A. G.; Hewitt, K. A., Effect of malaria on HIV-1 progression and transmission. *Lancet (London, England)* **2005**, 365 (9455), 196-7.
 107. Chirenda, J.; Murugasampillay, S., Malaria and HIV co-infection: available evidence, gaps and possible interventions. *The Central African journal of medicine* **2003**, 49 (5-6), 66-71.
 108. Patnaik, P., et al., Effects of HIV-1 serostatus, HIV-1 RNA concentration, and CD4 cell count on the incidence of malaria infection in a cohort of adults in rural Malawi. *J Infect Dis* **2005**, 192 (6), 984-91.
 109. Whitworth, J., et al., Effect of HIV-1 and increasing immunosuppression on malaria parasitaemia and clinical episodes in adults in rural Uganda: a cohort study. *Lancet* **2000**, 356 (9235), 1051-6.
 110. Hendriksen, I. C., et al., Diagnosis, clinical presentation, and in-hospital mortality of severe malaria in HIV-coinfected children and adults in Mozambique. *Clin Infect Dis* **2012**, 55 (8), 1144-53.
 111. Cohen, C., et al., Increased prevalence of severe malaria in HIV-infected adults in South Africa. *Clin Infect Dis* **2005**, 41 (11), 1631-7.
 112. Taylor, W. R., et al., Respiratory manifestations of malaria. *Chest* **2012**, 142 (2), 492-505.
 113. Kotepui, M., et al., Effects of malaria parasite density on blood cell parameters. *PloS one* **2015**, 10 (3), e0121057.
 114. Camacho, L. H., et al., The eosinophilic response and haematological recovery after treatment for *Plasmodium falciparum* malaria. *Trop Med Int Health* **1999**, 4 (7), 471-5.
 115. Vinetz, J. M., et al., *Plasmodium malariae* infection in an asymptomatic 74-year-old Greek woman with splenomegaly. *N Engl J Med* **1998**, 338 (6), 367-71.
 116. Allison, A. C., Protection afforded by sickle-cell trait against subtertian malarial infection. *Bmj* **1954**, 1, 290-294.
 117. Roberts, D. J.; Williams, T. N., Haemoglobinopathies and resistance to malaria. *Redox report : communications in free radical research* **2003**, 8 (5), 304-10.
 118. Hill, A. V. S., et al., Host genetic factors in resistance to malaria. 1998; p 445-455.
 119. Miller, L. H., et al., The resistance factor to *Plasmodium vivax* in blacks. The Duffy-blood-group genotype, FyFy. *The New England journal of medicine* **1976**, 295 (6), 302-4.
 120. Ferreira, A., et al., Sickle hemoglobin confers tolerance to *Plasmodium* infection. *Cell* **2011**, 145 (3), 398-409.
 121. Hebbel, R. P., Sickle hemoglobin instability: a mechanism for malarial protection. *Redox report : communications in free radical research* **2003**, 8 (5), 238-40.
 122. Cabrera, G., et al., The sickle cell trait is associated with enhanced immunoglobulin G antibody responses to *Plasmodium falciparum* variant surface antigens. *The Journal of infectious diseases* **2005**, 191 (10), 1631-8.
 123. Diallo, D. A., et al., A comparison of anemia in hemoglobin C and normal hemoglobin A children with *Plasmodium falciparum* malaria. *Acta tropica* **2004**, 90 (3), 295-9.
 124. Arie, T., et al., Hemoglobin C modulates the surface topography of *Plasmodium falciparum*-infected erythrocytes. *Journal of structural biology* **2005**, 150 (2), 163-9.
 125. Jarolim, P., et al., Deletion in erythrocyte band 3 gene in malaria-resistant Southeast Asian ovalocytosis. *Proceedings of the National Academy of Sciences of the United States of America* **1991**, 88 (24), 11022-6.
 126. Luzzi, G. A., et al., Surface antigen expression on *Plasmodium falciparum*-infected erythrocytes is

- modified in alpha- and beta-thalassemia. *The Journal of experimental medicine* **1991**, 173 (4), 785-91.
127. Glushakova, S., et al., Hemoglobinopathic erythrocytes affect the intraerythrocytic multiplication of *Plasmodium falciparum* in vitro. *J Infect Dis* **2014**, 210 (7), 1100-9.
 128. Howes, R. E., et al., *Plasmodium vivax* Transmission in Africa. *PLoS Negl Trop Dis* **2015**, 9 (11), e0004222.
 129. Lysenko, A. J.; Beljaev, A. E., An analysis of the geographical distribution of *Plasmodium ovale*. *Bull World Health Organ* **1969**, 40 (3), 383-94.
 130. Soe, S., et al., Premunition against *Plasmodium falciparum* in a malaria hyperendemic village in Myanmar. *Trans R Soc Trop Med Hyg* **2001**, 95 (1), 81-4.
 131. Perignon, J. L.; Druilhe, P., Immune mechanisms underlying the premunition against *Plasmodium falciparum* malaria. *Mem Inst Oswaldo Cruz* **1994**, 89 Suppl 2, 51-3.
 132. Biot, J.; Martin, M., [Contribution to the study of the premunition of malaria in the African adult. Apropos of 25 cases]. *Med Trop (Mars)* **1963**, 23, 106-11.
 133. Makkapati, V. V.; Rao, R. M., Ontology-based malaria parasite stage and species identification from peripheral blood smear images. *Conf Proc IEEE Eng Med Biol Soc* **2011**, 2011, 6138-41.
 134. Milne, L. M., et al., Accuracy of routine laboratory diagnosis of malaria in the United Kingdom. *Journal of clinical pathology* **1994**, 47 (8), 740-2.
 135. Wilson, M. L., Malaria rapid diagnostic tests. *Clin Infect Dis* **2012**, 54 (11), 1637-41.
 136. Visser, T., et al., Rapid diagnostic tests for malaria. *Bull World Health Organ* **2015**, 93 (12), 862-6.
 137. Mbabazi, P., et al., Accuracy of two malaria rapid diagnostic tests (RDTs) for initial diagnosis and treatment monitoring in a high transmission setting in Uganda. *Am J Trop Med Hyg* **2015**, 92 (3), 530-6.
 138. Gatton, M. L., et al., Pan-*Plasmodium* band sensitivity for *Plasmodium falciparum* detection in combination malaria rapid diagnostic tests and implications for clinical management. *Malar J* **2015**, 14, 115.
 139. Foster, D., et al., Evaluation of three rapid diagnostic tests for the detection of human infections with *Plasmodium knowlesi*. *Malar J* **2014**, 13, 60.
 140. Houze, S., et al., Combined deletions of pfhrp2 and pfhrp3 genes result in *Plasmodium falciparum* malaria false-negative rapid diagnostic test. *J Clin Microbiol* **2011**, 49 (7), 2694-6.
 141. Parr, J. B., et al., Streamlined, PCR-based testing for pfhrp2- and pfhrp3-negative *Plasmodium falciparum*. *Malar J* **2018**, 17 (1), 137.
 142. Bendezu, J., et al., Geographical distribution and genetic characterization of pfhrp2 negative *Plasmodium falciparum* parasites in the Peruvian Amazon. *PloS one* **2022**, 17 (11), e0273872.
 143. Proux, S., et al., Considerations on the use of nucleic acid-based amplification for malaria parasite detection. *Malar J* **2011**, 10, 323.
 144. Hopkins, H., et al., Highly sensitive detection of malaria parasitemia in a malaria-endemic setting: performance of a new loop-mediated isothermal amplification kit in a remote clinic in Uganda. *J Infect Dis* **2013**, 208 (4), 645-52.
 145. Cook, J., et al., Loop-mediated isothermal amplification (LAMP) for point-of-care detection of asymptomatic low-density malaria parasite carriers in Zanzibar. *Malar J* **2015**, 14, 43.
 146. Patel, J. C., et al., Field evaluation of a real-time fluorescence loop-mediated isothermal amplification assay, RealAmp, for the diagnosis of malaria in Thailand and India. *J Infect Dis* **2014**, 210 (8), 1180-7.
 147. Plowe, C. V., et al., Mutations in *Plasmodium falciparum* dihydrofolate reductase and dihydropteroate synthase and epidemiologic patterns of pyrimethamine-sulfadoxine use and resistance. *The Journal of infectious diseases* **1997**, 176 (6), 1590-6.
 148. Plowe, C. V., et al., *P. falciparum* dihydrofolate reductase and dihydropteroate synthase mutations: epidemiology and role in clinical resistance to antifolates. *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy* **1998**, 1 (6), 389-96.
 149. Sidhu, A. B. S., et al., Chloroquine resistance in *Plasmodium falciparum* malaria parasites conferred by pfcrt mutations. *Science (New York, N.Y.)* **2002**, 298 (5591), 210-3.
 150. Mita, T., et al., *Plasmodium falciparum* kelch 13: a potential molecular marker for tackling

- artemisinin-resistant malaria parasites. *Expert Rev Anti Infect Ther* **2016**, 14 (1), 125-35.
151. Arie, F., et al., A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* **2014**, 505 (7481), 50-5.
152. Beier, J. C., et al., Field evaluation of an enzyme-linked immunosorbent assay (ELISA) for *Plasmodium falciparum* sporozoite detection in anopheline mosquitoes from Kenya. *The American journal of tropical medicine and hygiene* **1987**, 36 (3), 459-68.
153. Baird, J. K., Effectiveness of antimalarial drugs. *The New England journal of medicine* **2005**, 352 (15), 1565-77.
154. Dondorp, A. M., et al., Artesunate versus quinine in the treatment of severe *falciparum* malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet* **2010**, 376 (9753), 1647-57.
155. Dondorp, A., et al., Artesunate versus quinine for treatment of severe *falciparum* malaria: a randomised trial. *Lancet* **2005**, 366 (9487), 717-25.
156. Smithuis, F., et al., Comparison of chloroquine, sulfadoxine/pyrimethamine, mefloquine and mefloquine-artesunate for the treatment of *falciparum* malaria in Kachin State, North Myanmar. *Tropical medicine & international health : TM & IH* **2004**, 9 (11), 1184-90.
157. Staedke, S. G., et al., Combination treatments for uncomplicated *falciparum* malaria in Kampala, Uganda: randomised clinical trial. *Lancet (London, England)* **2004**, 364 (9449), 1950-7.
158. CDC, Treatment with quinidine gluconate of persons with severe *Plasmodium falciparum* infection: discontinuation of parenteral quinine from CDC Drug Service. *MMWR Recomm Rep* **1991**, 40 (RR-4), 21.
159. Thomas, C. M., et al., Food and Drug Administration Approval of Artesunate for Severe Malaria: Enough to Achieve Best Practice? *Clin Infect Dis* **2023**, 76 (3), e864-e866.
160. Twomey, P. S., et al., Intravenous Artesunate for the Treatment of Severe and Complicated Malaria in the United States: Clinical Use Under an Investigational New Drug Protocol. *Ann Intern Med* **2015**, 163 (7), 498-506.
161. Berkley, J. A., et al., Bacteremia among children admitted to a rural hospital in Kenya. *N Engl J Med* **2005**, 352 (1), 39-47.
162. Tan, K. R., et al., Exchange transfusion for severe malaria: evidence base and literature review. *Clin Infect Dis* **2013**, 57 (7), 923-8.
163. Bojang, K. A., et al., Management of severe malarial anaemia in Gambian children. *Trans R Soc Trop Med Hyg* **1997**, 91 (5), 557-61.
164. English, M., et al., Blood transfusion for severe anaemia in children in a Kenyan hospital. *Lancet* **2002**, 359 (9305), 494-5.
165. Awasthi, G.; Das, A., Genetics of chloroquine-resistant malaria: a haplotypic view. *Mem Inst Oswaldo Cruz* **2013**, 108 (8), 947-61.
166. WHO, Guidelines for the treatment of malaria, 3rd ed. **2015**.
167. Zhu, L., et al., Artemisinin resistance in the malaria parasite, *Plasmodium falciparum*, originates from its initial transcriptional response. *Commun Biol* **2022**, 5 (1), 274.
168. Mihreteab, S., et al., Increasing Prevalence of Artemisinin-Resistant HRP2-Negative Malaria in Eritrea. *N Engl J Med* **2023**, 389 (13), 1191-1202.
169. Medical Letter on Drugs and Therapeutics. *Handbook of Antimicrobial Therapies* **2005**.
170. Miller, K. D., et al., Severe cutaneous reactions among American travelers using pyrimethamine-sulfadoxine (Fansidar) for malaria prophylaxis. *The American journal of tropical medicine and hygiene* **1986**, 35 (3), 451-8.
171. Deloron, P., et al., Sulfadoxine/pyrimethamine intermittent preventive treatment for malaria during pregnancy. *Emerg Infect Dis* **2010**, 16 (11), 1666-70.
172. Peters, P. J., et al., Safety and toxicity of sulfadoxine/pyrimethamine: implications for malaria prevention in pregnancy using intermittent preventive treatment. *Drug Saf* **2007**, 30 (6), 481-501.
173. Maguire, J. D., et al., Mefloquine is highly efficacious against chloroquine-resistant *Plasmodium vivax* malaria and *Plasmodium falciparum* malaria in Papua, Indonesia. *Clin Infect Dis* **2006**, 42 (8), 1067-72.
174. Looareesuwan, S., et al., Efficacy and safety of atovaquone/proguanil compared with mefloquine

- for treatment of acute *Plasmodium falciparum* malaria in Thailand. *Am J Trop Med Hyg* **1999**, 60 (4), 526-32.
175. Bustos, D. G., et al., Atovaquone-proguanil compared with chloroquine and chloroquine-sulfadoxine-pyrimethamine for treatment of acute *Plasmodium falciparum* malaria in the Philippines. *J Infect Dis* **1999**, 179 (6), 1587-90.
 176. Cui, L., et al., Antimalarial Drug Resistance: Literature Review and Activities and Findings of the ICEMR Network. *Am J Trop Med Hyg* **2015**, 93 (3 Suppl), 57-68.
 177. Rajapakse, S., et al., Tafenoquine for preventing relapse in people with *Plasmodium vivax* malaria. *Cochrane Database Syst Rev* **2015**, 2015 (4), CD010458.
 178. Anjum, M. U., et al., Single dose tafenoquine for preventing relapse in people with *Plasmodium vivax* malaria-an updated meta-analysis. *Travel Med Infect Dis* **2020**, 36, 101576.
 179. Rodrigo, C., et al., Tafenoquine for preventing relapse in people with *Plasmodium vivax* malaria. *Cochrane Database Syst Rev* **2020**, 9 (9), CD010458.
 180. Ritchie, E. C., et al., Psychiatric side effects of mefloquine: applications to forensic psychiatry. *J Am Acad Psychiatry Law* **2013**, 41 (2), 224-35.
 181. Hill, D. R., et al., Primaquine: report from CDC expert meeting on malaria chemoprophylaxis I. *Am J Trop Med Hyg* **2006**, 75 (3), 402-15.
 182. Porter, C. H.; Collins, F. H., Susceptibility of *Anopheles hermsi* to *Plasmodium vivax*. *The American journal of tropical medicine and hygiene* **1990**, 42 (5), 414-6.
 183. Dhiman, S.; Veer, V., Culminating anti-malaria efforts at long lasting insecticidal net? *Journal of infection and public health* **2014**, 7 (6), 457-464.
 184. Suh, P. F., et al., Impact of insecticide resistance on malaria vector competence: a literature review. *Malar J* **2023**, 22 (1), 19.
 185. Yang, G. G., et al., A Meta-Regression Analysis of the Effectiveness of Mosquito Nets for Malaria Control: The Value of Long-Lasting Insecticide Nets. *Int J Environ Res Public Health* **2018**, 15 (3).
 186. Smithuis, F. M., et al., The effect of insecticide-treated bed nets on the incidence and prevalence of malaria in children in an area of unstable seasonal transmission in western Myanmar. *Malar J* **2013**, 12, 363.
 187. James, A. A., Mosquito molecular genetics: the hands that feed bite back. *Science (New York, N.Y.)* **1992**, 257 (5066), 37-8.
 188. Gwadz, R. W., Genetic approaches to malaria control: how long the road? *The American journal of tropical medicine and hygiene* **1994**, 50 (6 Suppl), 116-25.
 189. Gantz, V. M., et al., Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*. *Proc Natl Acad Sci U S A* **2015**, 112 (49), E6736-43.
 190. Trager, W.; Jensen, J. B., Human malaria parasites in continuous culture. 1976. *J Parasitol* **2005**, 91 (3), 484-6.
 191. Gardner, M. J., et al., Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* **2002**, 419 (6906), 498-511.
 192. Hoffmann, S. L., Malaria Vaccine Development: A Multi-Immune Response Approach. ASM Press, Washington, D.C: **1996**.
 193. Miller, L. H.; Hoffman, S. L., Research toward vaccines against malaria. *Nature medicine* **1998**, 4 (5 Suppl), 520-4.
 194. Datto, M. S., et al., Efficacy and immunogenicity of R21/Matrix-M vaccine against clinical malaria after 2 years' follow-up in children in Burkina Faso: a phase 1/2b randomised controlled trial. *Lancet Infect Dis* **2022**, 22 (12), 1728-1736.
 195. White, M. T., et al., Immunogenicity of the RTS,S/AS01 malaria vaccine and implications for duration of vaccine efficacy: secondary analysis of data from a phase 3 randomised controlled trial. *Lancet Infect Dis* **2015**, 15 (12), 1450-8.
 196. www.census.gov/main/www/popclock.html.
 197. Bjorkman, A., et al., RTS,S/AS01 malaria vaccine-proven safe and effective? *Lancet Infect Dis* **2023**, 23 (8), e318-e322.

10. *Cryptosporidium parvum* and *C. hominis*

(Tyzzer 1929)

Cryptosporidium parvum

Pronunciation: \krip-tō-spōr-'i-dē-əm\
\pahr'vūm\

Introduction

The genus *Cryptosporidium* (\krip-tow-SPOR-i-dee-um\) comprises a very large group of closely related obligate intracellular parasites that cause transient diarrheal disease in most mammalian species worldwide, including humans. All are transmitted through fecally contaminated food and water.^{1,3} Most species have broad host ranges. Eight species have been shown to infect humans regularly: *C. parvum* (\PAAR-vum\), *C. hominis*, *C. meleagridis*, *C. felis*, *C. canis*, *C. muris*, and *Cryptosporidium* pig and deer species.⁴⁻¹⁰ Most human infections are caused by *C. parvum* and *C. hominis*, which infect sheep, cattle, birds, rodents, and non-human primates.¹¹ This chapter will concentrate on *C. parvum*, as that disease in humans caused by other related species results in a similar clinical picture.

In 1993, Milwaukee, Wisconsin, experienced the largest waterborne outbreak of diarrheal disease ever documented in the United States. Over 400,000 people suffered from infection with *C. parvum*.¹² In immunocompetent infected individuals, the most serious manifestation of infection is diarrhea of short duration, although sometimes severe. In contrast, infants, non-AIDS immunocompromised adults, and people living with HIV/AIDS often experience severe, protracted diarrhea, sometimes resulting in death.¹³ *C. parvum* can be grown axenically *in vitro* using monolayers of epithelial cells.^{14, 15} The genomes of *C. hominis* and *C. parvum* have been sequenced.^{11, 16, 17}

Historical Information

In 1907, Ernest Edward Tyzzer described *Cryptosporidium* based on histologic sections of mouse intestine, in which the parasites were observed attached to the epithelial cells.¹⁸ Several decades later, in 1955, D Slavin established that this protozoan caused diarrhea in turkeys.¹⁹ In 1976, F.A. Nime and coworkers described human diarrheal disease due to *Cryptosporidium*. That same year, J.L. Meisel and colleagues published the first case in an immunocompromised human host.^{20, 21} Currently, various species of *Cryptosporidium* are recognized as the cause of many cases of diarrhea in cows, calves, lambs, poultry, game birds, and humans.^{22, 23} In 2013, the Global Enteric Multicenter Study (GEMS) evaluated the etiology of diarrheal diseases in infants and young children using NAAT and identified *Cryptosporidium* as one of the four top causes of diarrhea in children younger than five years of age.²⁴

Life Cycle

Infection begins when the host ingests thick-walled sporulated oocysts (Fig. 10.1) containing four banana-shaped sporozoites.²⁵ A minimum of 30 oocysts is necessary to initi-

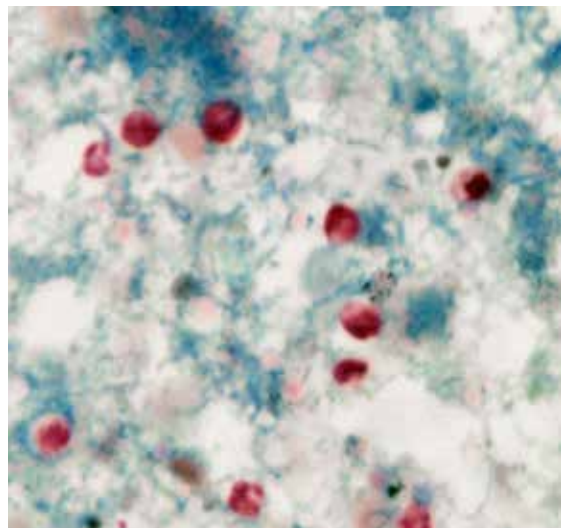


Figure 10.1. Oocysts of *Cryptosporidium parvum*. Cold acid fast stain. 5 μ m.

ate infection, while the dose required to infect 50% of healthy volunteers was 132 oocysts.²⁶

²⁷ An infected individual may release as many as a billion cysts during one infection.²⁸

The sporozoites excyst when the oocyst enters the small intestine. Little is known regarding the mechanism of excystment *in vivo*, except that a protein plug in the cyst wall blocks the escape route for sporozoites.²⁹ *In vitro*, excystment occurs after exposure to 37 °C or by pretreatment of purified oocysts with either sodium taurocholate and trypsin or with sodium hypochlorite (bleach) alone, followed by introduction into culture medium.³⁰ Oocysts treated with bleach can be inhibited from excysting by exposure to human α -1-anti-trypsin inhibitors or inhibitors of arginine aminopeptidase.^{31, 32} Like other enteric parasites with resistant outer structures (e.g., eggs of helminths and cysts of *Giardia* and *Entamoeba*), alteration of the outer surface may be a prerequisite for the organism to receive environmental cues, triggering the synthesis of enzymes of parasite

origin required for emergence.

Sporozoites attach to the surface of epithelial cells (Fig. 10.2), most likely aided by numerous proteins secreted from their rhoptries (\rhoptrīs\') and micronemes. A monoclonal antibody, designated 3E2, binds solely to the apical complex of the organism (the region where microneme and rhoptry-specific proteins exit from the parasite) and inhibits invasion *in vitro*.³³ On Western Blot analysis, this antibody recognizes numerous epitopes, ranging from 46 kDa to 1300 kDa. Furthermore, when employed in competitive inhibition studies, a purified microneme-specific mucin-like 900 kDa glycoprotein can prevent invading parasites from attaching to their target cells.³⁴

After the sporozoite attaches to the cell surface, most likely mediated by thrombospondins and related adhesive proteins, microvilli in the area immediately adjacent to the parasite fuse and elongate, enveloping the parasite to create a unique intracellular environment

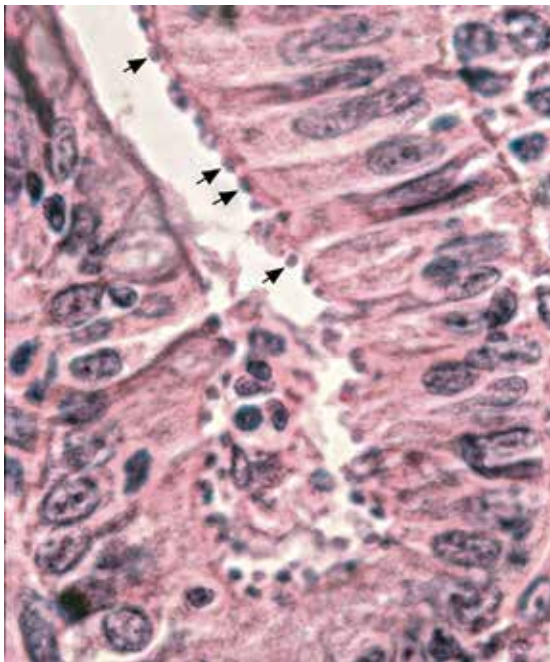


Figure 10.2. Histologic section of small intestine of patient suffering from HIV/AIDS, infected with *C. parvum* (arrows). Courtesy J. Lefkowitz.

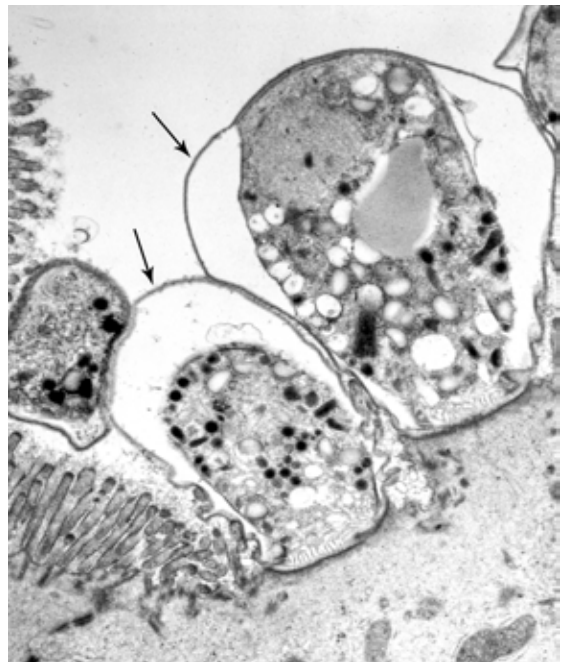
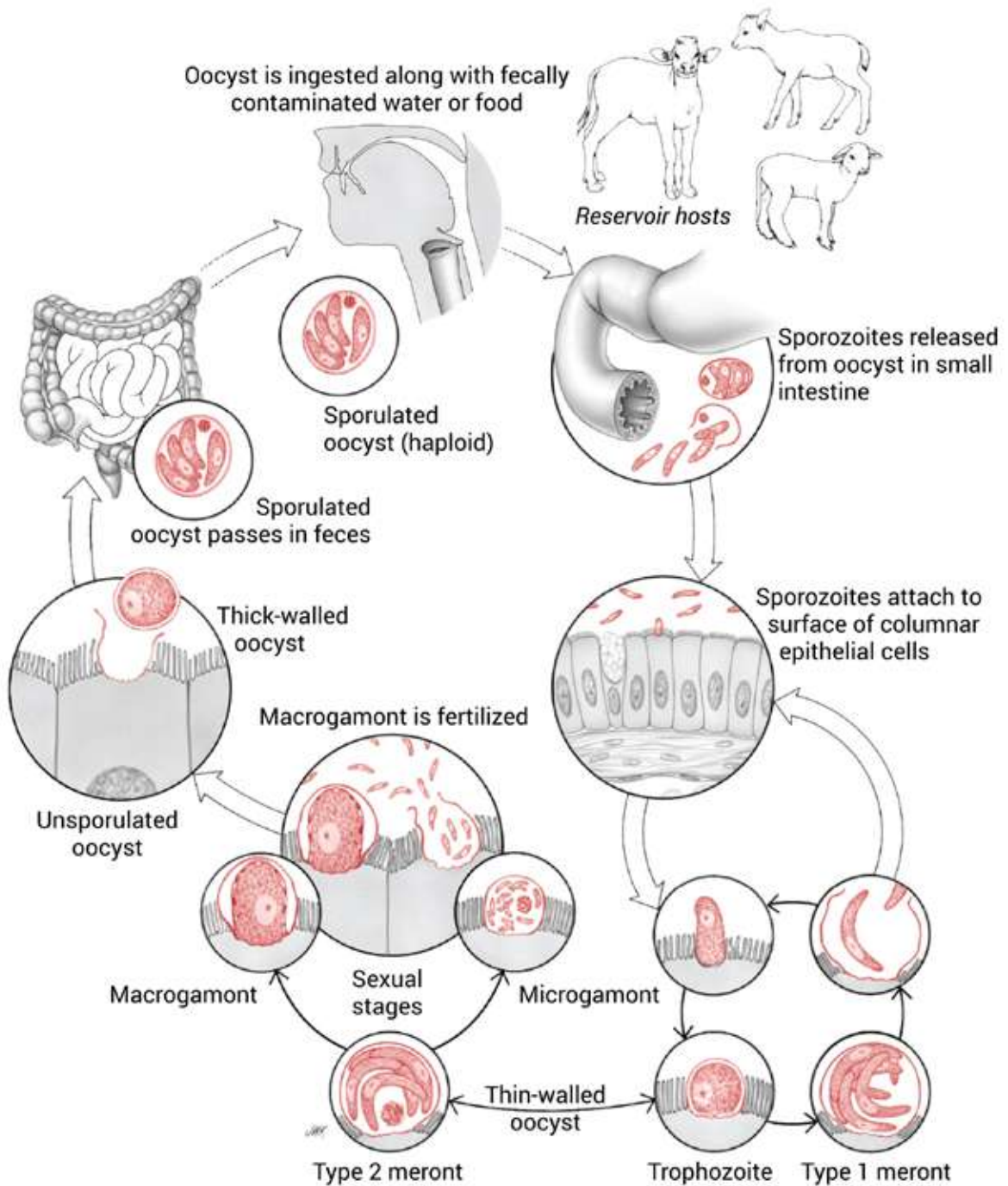


Figure 10.3. Transmission EM of *C. parvum*. Note microvillus-derived membranes encasing parasites (arrows). Courtesy J. Lefkowitz.

Cryptosporidium parvum



(Fig. 10.3).³⁵ Apical end-associated secreted proteins may also trigger this event. A specialized membrane structure develops at the interface between the parasite and the host cell. Nutrients are thought to pass through this region since parasite-specific ABC transporters have been identified using immunofluorescent monoclonal antibodies.³⁶ The parasite then induces alterations in the gene expression of the invaded host cell, eliciting the upregulation of osteoprotegerin, a TNF family member known to inhibit apoptosis.³⁷ Such a strategy would favor the long-term survival of the parasite until it can complete its development and move to the next stage in its life cycle.³⁸ The sporozoite differentiates into the type I meront (Fig. 10.4), and division ensues, producing four haploid merozoites.

The merozoites are released and attach to new epithelial cells, now differentiating into Type II meronts. Macrogamonts and microgamonts (pre-sex cells analogous to the gametocytes of *Plasmodia*) are produced inside these newly invaded cells. After their release, microgamonts fuse with macrogamonts, forming thick-walled zygotes called oocysts. This stage sporulates within the large intestine, producing four haploid infectious sporozoites. Oocysts can also be thin-walled. In this case, they sporulate and excyst within

the same host, producing an autoinfection that may endure for months to years. Even in these cases, thick-walled oocysts are produced, as well.

Thick-walled oocysts pass out in feces and can infect another host. This type of oocyst is environmentally resistant and can remain viable for months to years in soil, given optimum moisture conditions.³⁹

Cellular and Molecular Pathogenesis

One of the most perplexing and frustrating aspects of the biology of *C. parvum* has been its lack of response to a wide variety of drugs.⁴⁰⁻⁴² While attached to epithelial cells, the altered microvillus-derived membrane complex surrounding the parasites has proven highly impermeable to many chemotherapeutic agents. That is why speculation favors the entry of nutrients through the attachment zone between the parasite and the host cell's surface. The fact that ABC transporters have been identified in this region is further indirect evidence in support of this hypothesis. Cellular or molecular events that result in the alteration of microvilli at the site of attachment have attracted the attention of some research groups.⁴³ Apparently, Cdc42 (a GTPase) and actin are recruited to the site of attachment early on in the process.⁴¹ Actin then aggregates, forming a platform on top of which the organism then elaborates its complex of membranes. Much more must be learned about the mechanism(s) of nutrient acquisition by *C. parvum* before rational drug design aimed at interfering with this process can evolve.

Now that the genome has been fully sequenced, its pathogenesis is being deciphered.^{16, 17} More than 25 possible virulence

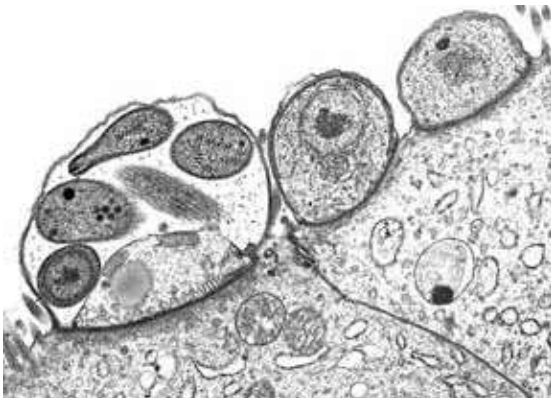


Figure 10.4. Transmission EM of *C. parvum* meronts. Courtesy M. Belosevic.

factors have been isolated, and their specific roles in causing clinical illness are being examined.²³ Virulence factors may involve excystation, adhesion, locomotion, invasion, intracellular multiplication, survival, and host cell damage.²³ Several factors, such as phospholipases, proteases, and hemolysins, appear to play a part in causing direct damage to host cells.⁴⁴ Pro-inflammatory cytokines such as interleukin-8 are possibly involved in addition to microbial virulence factors.⁴⁵

Protection against the primary infection develops in individuals without compromised immune systems. At least two classes of antibodies, IgA and IgG, and several cellular-based immune mechanisms are thought to be important in eliminating the parasite from the gut tract. However, the precise mechanisms responsible for this have yet to be determined.^{27, 46, 47} Healthy human volunteers whose anti-*C. parvum* IgG levels were already present (exposed, immune), required a higher dose of oocysts to become infected, and developed fewer symptoms than their non-exposed (non-immune) counterparts.²⁷ Patients living in areas endemic to *C. parvum* have milder symptoms with repeated infections.⁴⁸ Experimental infections in multiple strains of inbred mice show that interleukin-12, interferon- γ , and perhaps β -defensins, peptides chemically related to magainins, act in conjunction to protect against challenge infection.^{47, 49-53} Calves fed irradiated oocysts of *C. parvum* were protected from a challenge infection, implying that protection-inducing antigens are present in this stage of the parasite.⁵⁴ Patients who have AIDS may develop an antibody response that is measurable in both serum and intestinal secretions, but they are unable to clear their infection.⁵⁵⁻⁵⁷ In underserved regions of the tropics, many children born HIV-positive are dying from this opportunistic infection.⁵⁸

Clinical Disease

Infection can be initiated in several ways: ingesting contaminated water, directly from an infected person or animal, consuming tainted food, or rarely through aerosol.⁵⁹ The disease can vary from asymptomatic infection to mild or profuse watery diarrhea in immunocompetent individuals. Upper abdominal cramps, anorexia, nausea, weight loss, and vomiting are common features of the acute stage of the infection.⁶⁰ The Severity of disease does not appear to correlate with the intensity of exposure.²⁶ In those who have recovered, a second infecting dose of oocysts may be asymptomatic or induce only mild, transient diarrhea. Cryptosporidiosis in an immunocompetent host is self-limited, lasting up to 2 weeks, but in some individuals, may persist for extended periods. In others, diarrhea may be severe, with several liters daily and even persistent diarrhea impacting nutrition and growth.⁶¹

Children are the most severely affected group, as the diarrhea lasts longer, and there is usually associated weight loss.⁶² With the introduction of molecular testing, cryptosporidiosis is now recognized as one of the four leading causes of diarrhea in children under five years of age.²⁴ Those undergoing cancer chemotherapies can suffer severe disease with protracted, life-threatening diarrhea and significant weight loss.⁶³

Cryptosporidiosis in patients with AIDS is chronic, lasting months and even years, during which patients can lose more than three liters of fluid each day and are in significant danger of dying; the case fatality rate can be as high as 50%. Death is usually a result of associated conditions, such as malnutrition or superinfection with other pathogens. Extra-intestinal infection in the bile duct can cause acalculous biliary disease.

Diagnosis (see Clinical Appendix)

Diagnosis can be made by identification of acid fast-stained oocysts seen on microscopic examination of stool (Fig. 10.1), stool antigen testing, directed PCR testing or multiplex NAAT.^{64,65} Stool microscopy is the least sensitive test, with sensitivities as low as 30% with single stool examinations.⁶⁶ Oocysts can be isolated from stool by flotation in a sugar solution and then stained by acid-fast methods.⁶⁷ PCR testing is highly sensitive and can identify specific genotypes, important in outbreaks and epidemiological investigations.⁶⁸ ⁶⁹ Antigen tests using monoclonal antibodies have high sensitivity, are easy to perform, and can be used in feces and tissue specimens.⁷⁰ The introduction of multiplex NAAT testing has helped to increase the number of cases of infectious diarrhea, which is now recognized to be due to *Cryptosporidium* spp.²⁴ Multiplex stool NAAT is now a highly sensitive and commercially available diagnostic option for diagnosing cryptosporidiosis.⁷¹

Treatment (see Clinical Appendix)

Treatment of cryptosporidiosis is based on the features of the infected host. In immunologically healthy children, nitazoxanide is the drug of choice based on studies demonstrating early clinical improvement, earlier resolution of diarrhea, and improved termination of oocyst shedding.^{40, 72-74} HIV-1 infected patients are best approached with the initiation of HAART to reconstitute their immune systems. All patients should receive

supportive care with oral rehydration when possible and intravenous therapy if required. Clinical trials with rifamycins, azalides, and paromomycin have demonstrated no apparent benefit, illustrating the difficulty of treating acute or established diseases.⁷⁵

Prevention and Control

Good hygiene is essential to decreasing one's risk of exposure. Controlling and preventing infection due to *C. parvum* is challenging when the source of the outbreak is unknown.⁷⁶ In waterborne epidemics, managing watersheds is the long-term solution in situations where the water supply is not filtered.^{12, 77} Filtering drinking water is usually adequate, but deterioration of filtration equipment and lack of proper maintenance can erode any progress in controlling waterborne infections. Boiling contaminated water is another option for prevention. Chlorination of water supplies is ineffective against the oocyst, but ozonation kills this stage.^{78, 79} In agricultural settings, creating vegetative barriers to curtail the spread of oocysts is effective.⁸⁰ Surveillance is critical to keeping public water supplies free of pathogens with environmentally resistant stages (e.g., *Giardia lamblia*, *Entamoeba histolytica*, *C. parvum*). PCR-based testing facilitates continuous monitoring of water supplies for *C. parvum*.⁸¹ Urban and suburban pet stores and petting zoos for children are other sources of infection that, until recently, have received little attention.⁸²

References

1. Laberge, I.; Griffiths, M. W., Prevalence, detection and control of *Cryptosporidium parvum* in food. *International journal of food microbiology* **1996**, 32 (1-2), 1-26.
2. Keusch, G. T., et al., *Cryptosporidia*--who is at risk? *Schweizerische medizinische Wochenschrift* **1995**, 125 (18), 899-908.
3. Guerrant, R. L., Cryptosporidiosis: an emerging, highly infectious threat. *Emerging infectious*

- diseases **1997**, 3 (1), 51-7.
4. Katsumata, T., et al., Short report: possible *Cryptosporidium muris* infection in humans. *The American journal of tropical medicine and hygiene* **2000**, 62 (1), 70-2.
 5. Ong, C. S. L., et al., Novel *Cryptosporidium* genotypes in sporadic cryptosporidiosis cases: first report of human infections with a cervine genotype. *Emerging infectious diseases* **2002**, 8 (3), 263-8.
 6. Pedraza-Diaz, S., et al., The identification and characterisation of an unusual genotype of *Cryptosporidium* from human faeces as *Cryptosporidium meleagridis*. *FEMS Microbiol Letters* **2000**, 189, 189-194.
 7. Pedraza-Diaz, S.; Amar, C., Unusual *Cryptosporidium* species recovered from human faeces: first description of *Cryptosporidium felis* and *Cryptosporidium* 'dog type' from patients in England. *J Med Microbiol* **2001**, 50, 293-296.
 8. Pieniazek, N. J., et al., New cryptosporidium genotypes in HIV-infected persons. *Emerging infectious diseases* **1999**, 5 (3), 444-9.
 9. Xiao, L., et al., Identification of the *Cryptosporidium* pig genotype in a human patient. *The Journal of infectious diseases* **2002**, 185 (12), 1846-8.
 10. Xiao, L., et al., Identification of 5 types of *Cryptosporidium* parasites in children in Lima, Peru. *The Journal of infectious diseases* **2001**, 183 (3), 492-7.
 11. Hadfield, S. J., et al., Generation of whole genome sequences of new *Cryptosporidium hominis* and *Cryptosporidium parvum* isolates directly from stool samples. *BMC Genomics* **2015**, 16, 650.
 12. Kramer, M. H., et al., Surveillance for waterborne-disease outbreaks--United States, 1993-1994. *MMWR. CDC surveillance summaries : Morbidity and mortality weekly report. CDC surveillance summaries / Centers for Disease Control* **1996**, 45 (1), 1-33.
 13. Farthing, M. J., et al., Recently recognised microbial enteropathies and HIV infection. *The Journal of antimicrobial chemotherapy* **1996**, 37 Suppl B, 61-70.
 14. Current, W. L.; Reese, N. C., A comparison of endogenous development of three isolates of *Cryptosporidium* in suckling mice. *The Journal of protozoology* **1986**, 33 (1), 98-108.
 15. Meloni, B. P.; Thompson, R. C., Simplified methods for obtaining purified oocysts from mice and for growing *Cryptosporidium parvum* in vitro. *The Journal of parasitology* **1996**, 82 (5), 757-62.
 16. Xu, P., et al., The genome of *Cryptosporidium hominis*. *Nature* **2004**, 431 (7012), 1107-12.
 17. Abrahamsen, M. S., et al., Complete genome sequence of the apicomplexan, *Cryptosporidium parvum*. *Science (New York, N.Y.)* **2004**, 304 (5669), 441-5.
 18. Tyzzer, E. E., A Study of Heredity in Relation to the Development of tumors in Mice. *The Journal of medical research* **1907**, 17 (2), 199-211.
 19. Slavin, D.; J., *Cryptosporidium meleagridis* (sp. nov.). *Pathol* **1955**, 65, 262-266.
 20. Nime, F. A., et al., Acute enterocolitis in a human being infected with the protozoan *Cryptosporidium*. *Gastroenterology* **1976**, 70 (4), 592-8.
 21. Meisel, J. L., et al., Overwhelming watery diarrhea associated with a *Cryptosporidium* in an immunosuppressed patient. *Gastroenterology* **1976**, 70 (6), 1156-60.
 22. de Graaf, D. C., et al., A review of the importance of cryptosporidiosis in farm animals. *Int J Parasitol* **1999**, 29 (8), 1269-87.
 23. Bouzid, M., et al., *Cryptosporidium* pathogenicity and virulence. *Clin Microbiol Rev* **2013**, 26 (1), 115-34.
 24. Kotloff, K. L., et al., Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* **2013**, 382 (9888), 209-22.
 25. Fayer, R., *Cryptosporidium*: a water-borne zoonotic parasite. *Veterinary parasitology* **2004**, 126 (1-2), 37-56.
 26. DuPont, H. L., et al., The infectivity of *Cryptosporidium parvum* in healthy volunteers. *The New England journal of medicine* **1995**, 332 (13), 855-9.

27. Chappell, C. L., et al., Infectivity of *Cryptosporidium parvum* in healthy adults with pre-existing anti-*C. parvum* serum immunoglobulin G. *The American journal of tropical medicine and hygiene* **1999**, 60 (1), 157-64.
28. Chappell, C. L., et al., *Cryptosporidium parvum*: intensity of infection and oocyst excretion patterns in healthy volunteers. *J Infect Dis* **1996**, 173 (1), 232-6.
29. Neuman, D., et al., Structural and functional characteristics of rhenium clusters derived from redox chemistry of the triangular [ReIII3(μ -Cl)₃] core unit. *Inorganic chemistry* **2000**, 39 (24), 5530-7.
30. Forney, J. R., et al., Antagonistic effect of human alpha-1-antitrypsin on excystation of *Cryptosporidium parvum* oocysts. *The Journal of parasitology* **1997**, 83 (4), 771-4.
31. Okhuysen, P. C., et al., *Cryptosporidium parvum* metalloaminopeptidase inhibitors prevent *in vitro* excystation. *Antimicrobial agents and chemotherapy* **1996**, 40 (12), 2781-4.
32. Langer, R. C.; Riggs, M. W., *Cryptosporidium parvum* apical complex glycoprotein CSL contains a sporozoite ligand for intestinal epithelial cells. *Infection and immunity* **1999**, 67 (10), 5282-91.
33. Riggs, M. W., et al., Protective monoclonal antibody defines a circumsporozoite-like glycoprotein exoantigen of *Cryptosporidium parvum* sporozoites and merozoites. *Journal of immunology (Baltimore, Md. : 1950)* **1997**, 158 (4), 1787-95.
34. Barnes, D. A., et al., A novel multi-domain mucin-like glycoprotein of *Cryptosporidium parvum* mediates invasion. *Mol Biochem Parasitol* 9693 **1998**, 110, 1-2.
35. Wanyiri, J.; Ward, H., Molecular basis of *Cryptosporidium*-host cell interactions: recent advances and future prospects. *Future Microbiol* **2006**, 1 (2), 201-8.
36. Zapata, F., et al., The *Cryptosporidium parvum* ABC protein family. *Molecular and biochemical parasitology* **2002**, 120 (1), 157-61.
37. Di Genova, B. M.; Tonelli, R. R., Infection Strategies of Intestinal Parasite Pathogens and Host Cell Responses. *Front Microbiol* **2016**, 7, 256.
38. Castellanos-Gonzalez, A., et al., *Cryptosporidium* infection of human intestinal epithelial cells increases expression of osteoprotegerin: a novel mechanism for evasion of host defenses. *J Infect Dis* **2008**, 197 (6), 916-23.
39. Brasseur, P., et al., Viability of *Cryptosporidium parvum* oocysts in natural waters. *Folia parasitologica* **1998**, 45 (2), 113-6.
40. Smith, H. V.; Corcoran, G. D., New drugs and treatment for cryptosporidiosis. *Current opinion in infectious diseases* **2004**, 17 (6), 557-64.
41. Chen, X.-M., et al., Cdc42 and the actin-related protein/neural Wiskott-Aldrich syndrome protein network mediate cellular invasion by *Cryptosporidium parvum*. *Infection and immunity* **2004**, 72 (5), 3011-21.
42. Sparks, H., et al., Treatment of *Cryptosporidium*: What We Know, Gaps, and the Way Forward. *Curr Trop Med Rep* **2015**, 2 (3), 181-187.
43. Huang, B. Q., et al., *Cryptosporidium parvum* attachment to and internalization by human biliary epithelia *in vitro*: a morphologic study. *The Journal of parasitology* **2004**, 90 (2), 212-21.
44. Okhuysen, P. C.; Chappell, C. L., *Cryptosporidium* virulence determinants--are we there yet? *Int J Parasitol* **2002**, 32 (5), 517-25.
45. Kirkpatrick, B. D., et al., Childhood cryptosporidiosis is associated with a persistent systemic inflammatory response. *Clin Infect Dis* **2006**, 43 (5), 604-8.
46. Jenkins, M. C., et al., Hyperimmune bovine colostrum specific for recombinant *Cryptosporidium parvum* antigen confers partial protection against cryptosporidiosis in immunosuppressed adult mice. *Vaccine* **1999**, 17 (19), 2453-60.
47. McDonald, V., Host cell-mediated responses to infection with *Cryptosporidium*. *Parasite immunology* **2000**, 22 (12), 597-604.
48. Okhuysen, P. C., et al., Susceptibility and serologic response of healthy adults to reinfection with *Cryptosporidium parvum*. *Infect Immun* **1998**, 66 (2), 441-3.
49. Urban, J. F., et al., IL-12 protects immunocompetent and immunodeficient neonatal mice against infection with *Cryptosporidium parvum*. *J Immunol* **1998**, 156 (1), 263-268.

50. Tarver, A. P., et al., Enteric beta-defensin: molecular cloning and characterization of a gene with inducible intestinal epithelial cell expression associated with *Cryptosporidium parvum* infection. *Infection and immunity* **1998**, 66 (3), 1045-56.
51. Giacometti, A., et al., In-vitro activity of polycationic peptides against *Cryptosporidium parvum*, *Pneumocystis carinii* and yeast clinical isolates. *The Journal of antimicrobial chemotherapy* **1999**, 44 (3), 403-6.
52. Ludtke, S. J., et al., Membrane pores induced by magainin. *Biochemistry* **1996**, 35 (43), 13723-8.
53. Huang, H. W., Peptide-lipid interactions and mechanisms of antimicrobial peptides. *Novartis Foundation symposium* **1999**, 225, 188-200; discussion 200.
54. Jenkins, M., et al., Protection of calves against cryptosporidiosis by oral inoculation with gamma-irradiated *Cryptosporidium parvum* oocysts. *The Journal of parasitology* **2004**, 90 (5), 1178-80.
55. Benhamou, Y., et al., Inefficacy of intestinal secretory immune response to *Cryptosporidium* in acquired immunodeficiency syndrome. *Gastroenterology* **1995**, 108 (3), 627-35.
56. Cozon, G., et al., Secretory IgA antibodies to *Cryptosporidium parvum* in AIDS patients with chronic cryptosporidiosis. *J Infect Dis* **1994**, 169 (3), 696-9.
57. Ravera, M., et al., Prevalence of *Cryptosporidium parvum* in AIDS and immunocompetent patients in Uganda. *Int J STD AIDS* **1994**, 5 (4), 302-3.
58. Guarino, A., et al., Management of gastrointestinal disorders in children with HIV infection. *Paediatric drugs* **2004**, 6 (6), 347-62.
59. Putignani, L.; Menichella, D., Global distribution, public health and clinical impact of the protozoan pathogen *Cryptosporidium*. *Interdiscip Perspect Infect Dis* **2010**, 2010.
60. Farthing, M. J., Clinical aspects of human cryptosporidiosis. *Contributions to microbiology* **2000**, 6, 50-74.
61. Checkley, W., et al., Effects of *Cryptosporidium parvum* infection in Peruvian children: growth faltering and subsequent catch-up growth. *Am J Epidemiol* **1998**, 148 (5), 497-506.
62. Cicirello, H. G., et al., Cryptosporidiosis in children during a massive waterborne outbreak in Milwaukee, Wisconsin: clinical, laboratory and epidemiologic findings. *Epidemiology and infection* **1997**, 119 (1), 53-60.
63. Burgner, D., et al., Epidemiology of *Cryptosporidium parvum* in symptomatic paediatric oncology patients. *Journal of paediatrics and child health* **1999**, 35 (3), 300-2.
64. Blackman, E., et al., Cryptosporidiosis in HIV-infected patients: diagnostic sensitivity of stool examination, based on number of specimens submitted. *The American journal of gastroenterology* **1997**, 92 (3), 451-3.
65. Coupe, S., et al., Detection of *Cryptosporidium* and identification to the species level by nested PCR and restriction fragment length polymorphism. *Journal of clinical microbiology* **2005**, 43 (3), 1017-23.
66. Blanshard, C., et al., Cryptosporidiosis in HIV-seropositive patients. *Q J Med* **1992**, 85 (307-308), 813-23.
67. Ignatius, R., et al., Efficacy of different methods for detection of low *Cryptosporidium parvum* oocyst numbers or antigen concentrations in stool specimens. *Eur J*.
68. Morgan, U. M., et al., Comparison of PCR and microscopy for detection of *Cryptosporidium parvum* in human fecal specimens: clinical trial. *J Clin Microbiol* **1998**, 36 (4), 995-8.
69. Morgan, U. M.; Thompson, R. C., PCR detection of cryptosporidium: the way forward? *Parasitol Today* **1998**, 14 (6), 241-5.
70. Garcia, L. S.; Shimizu, R. Y., Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens. *J Clin Microbiol* **1997**, 35 (6), 1526-9.
71. Buss, S. N., et al., Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. *J Clin Microbiol* **2015**, 53 (3), 915-25.
72. Fox, L. M.; Saravolatz, L. D., Nitazoxanide: a new thiazolide antiparasitic agent. *Clin Inf Dis* **2005**, 40 (8), 1173-80.
73. Rossignol, J. F., et al., Treatment of diarrhea caused by *Cryptosporidium parvum*: a prospective randomized, double-blind, placebo-controlled study of Nitazoxanide. *J Infect Dis* **2001**, 184 (1), 103-6.

74. Rossignol, J. F., et al., Effect of nitazoxanide in diarrhea and enteritis caused by *Cryptosporidium* species. *Clin Gastroenterol Hepatol* **2006**, *4* (3), 320-4.
75. Checkley, W., et al., A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium. *Lancet Infect Dis* **2015**, *15* (1), 85-94.
76. Steiner, T. S., et al., Protozoal agents: what are the dangers for the public water supply? *Annual review of medicine* **1997**, *48*, 329-40.
77. Clancy, J. L., et al., UV light inactivation of *Cryptosporidium* oocysts. *Water Works Assoc* **1998**, *90*, 92-102.
78. Gyurek, L. L., et al., Ozone inactivation kinetics of *Cryptosporidium parvum* in phosphate buffer. *Engine* **2000**, *125*, 913-924.
79. Tate, K. W., et al., Efficacy of vegetated buffer strips for retaining *Cryptosporidium parvum*. *Journal of environmental quality* **2004**, *33* (6), 2243-51.
80. Hallier-Soulier, S.; Guillot, E., An immunomagnetic separation polymerase chain reaction assay for rapid and ultra-sensitive detection of *Cryptosporidium parvum* in drinking water. *FEMS microbiology letters* **1999**, *176* (2), 285-9.
81. Itoh, N., et al., Molecular detection and characterization of *Cryptosporidium* species in household dogs, pet shop puppies, and dogs kept in a school of veterinary nursing in Japan. *Vet Parasitol* **2014**, *200* (3-4), 284-8.

11. *Toxoplasma gondii*

(Nicolle and Manceaux 1908)

Pronunciation: \tāk-sə-'plaz-mə\\
gon'dē-ī\

Introduction

Toxoplasma gondii (\TOK-so-plaz-ma\\gon-DEE-eye\)) is an obligate intracellular parasite with a worldwide distribution. Its biology is similar to other members of the phylum Apicomplexa, which includes the *Plasmodium* spp., *Cystoisospora* spp., *Babesia* spp., *Cyclospora* spp., and *Cryptosporidium* spp.. *Toxoplasma gondii* infects most warm-blooded animals, including domesticated and wild birds.¹ Although reliable information is not available in many parts of the world, estimates based on seroprevalence studies from Brazil and parts of Indonesia suggest that the majority of individuals living in these countries are infected.² *T. gondii* is one of the most successful parasites on earth. It has even emerged as a serious pathogen of some marine mammals, such as sea otters.³ It can remain alive as a dormant infection for the host's life. When immunity breaks down, it can reactivate, often with clinical consequences. In this regard, *Toxoplasma* behaves similarly to other infectious agents whose reproduction is held in check by host-acquired protective immune responses (e.g., herpes simplex virus, *Mycobacterium tuberculosis*).

T. gondii is easily cultured and can be experimentally transfected, facilitating studies on its genetics, cellular, and molecular biology.^{4,6} Its genome is now sequenced.^{7,8} *Toxoplasma* is usually acquired through ingesting infected raw or undercooked meats, but several outbreaks were traced back to drinking water

supplies contaminated with oocysts.^{9, 10} The domestic cat and other feline species serve as the definitive host, harboring the sexual stages of the parasite.

In immunocompetent humans, infection rarely leads to serious illness. In contrast, when *T. gondii* infects immunocompromised individuals or when a previously acquired infection is reactivated, the clinical disease that follows can often be life-threatening.¹¹ Congenital infection also occurs, occasionally leading to devastating pathological consequences for the developing fetus.

Historical Information

In 1908, Charles Nicolle and Louis Manceaux described the organism they isolated from the gondi (*Ctenodactylus gondii*), a gerbil-like desert-inhabiting mammal.¹² In the same year, Alfonso Splendore, who was working in Brazil, described the identical parasite, which he identified in the tissues of rabbits.¹³ They published their results simultaneously but in different publications, so neither was aware of the other's findings. In 1923, Josef Janku described the congenital manifestations of the infection, which he accurately characterized as causing hydrocephalus and chorioretinitis.¹⁴ Janku could not isolate the organism from the brains of its victims. Several years later, in 1939, Abner Wolf and colleagues confirmed Janku's clinical description and experimentally transferred the infection from infected brain tissue to mice and rabbits.¹⁵ In 1941, H. Pinkerton, W.R. Henderson, and Alfred Sabin independently described cases of adult-acquired toxoplasmosis.^{16, 17} In 1970, John Frenkel and colleagues identified the sexual stages of the life cycle working in cats, as did William M. Hutchinson and co-workers in that same year.^{18, 19}



Figure 11.1. Sporulated oocysts of *Toxoplasma gondii*. 12 μm .

Life Cycle

Definitive Host Cycle

Felidae are the definitive host for *T. gondii*.⁴ Domestic cats acquire the infection in one of three ways: 1. ingesting oocysts in contaminated cat feces (Fig. 11.1), 2. ingesting the tissue cysts (Fig. 11.2) harbored by infected prey (e.g., mice, rats, rabbits, squirrels), or 3. ingesting tissue cysts in the form of leftover bits of ground meats (particularly pork and lamb). The cycle usually involves cats and rodents or birds. Rodents acquire the asexual tissue cyst stage of the parasite by ingesting food or water tainted with cat feces containing oocysts. Although feces is likely the primary source of infectious oocysts, this stage may be present in other feline body fluids such as saliva, milk, sputum, tears, semen, and urine.^{20, 21}

T. gondii can follow two development paths: the enteric and the extra-intestinal. It is only in Felidae that the enteric pathway with the sexual stage can occur. Infection in cats is usually initiated when they consume tissue cysts. The tissue cyst contains hundreds of infec-

tious units, termed bradyzoites. When the cat eats this stage, the cyst wall becomes partially digested in the stomach and fully ruptures in the small intestine, releasing its complement of bradyzoites. This stage invades epithelial cells, developing into merozoites. The intracellular merozoite undergoes multiple cycles of division by a process termed endodyogeny (uncountable biology). Finally, the sheer number of parasites overwhelms the cell, and the merozoites are released into the lumen of the small intestine. Each merozoite can infect other epithelial cells, continuing the infection.

Alternatively, merozoites can develop into gametocytes (male and female). The two sexual forms fuse, forming an oocyst that passes out with the fecal mass. This completes the portion of the enteric cycle that occurs inside the Felidae. Oocysts sporulate outside the host, producing haploid sporozoites, the infectious stage for the intermediate host or another cat. Cats do not develop a high enough level of protective immunity after exposure to a primary infection to prevent reinfection with the oocyst stage. Long-term protection can be induced in experimental situations, giving hope for the eventual development of an effective vaccine.²² There currently is a live vaccine using a mutant strain of *T. gondii* that reduces cyst development in sheep and inhibits the sexual development of *T. gondii* in the feline intestinal tract.²³

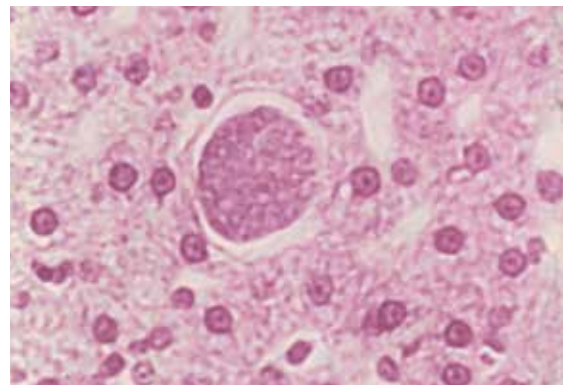


Figure 11.2. Pseudocyst of *T. gondii* in liver biopsy.



Figure 11.3. Tachyzoites of *T. gondii* in parasitophorous vacuoles of infected fibroblast.

Domestic and feral cats are implicated as the hosts most commonly responsible for transmitting the infection to farm animals (e.g., cattle, pigs, sheep, dogs, etc.). In addition, as will be described under clinical aspects, house cats may be considered a health hazard to pregnant women and their fetuses.

Intermediate Host Cycle

The oocyst stage contains the infectious sporozoites. Ingestion of this stage leads to infection in mammals and birds. Sporozoites are released by oocyst exposure to digestive enzymes in the small intestine. The freed parasites penetrate the intestinal wall and are taken up by macrophages. *T. gondii* can now follow the extra-intestinal pathway.

The organisms are called tachyzoites once inside a cell (Fig. 11.3). This stage resides in its membrane-bound parasitophorous vacuole.²⁴ Infected cells cannot destroy tachyzoites because *T. gondii* inhibits the fusion process between the lysosomal vessels and this specialized intracellular niche (Figs. 11.4, 11.5).²⁵ Replication occurs inside the macrophages, and parasites are passively carried to all parts of the body. Approximately 8–20 tachyzoites are produced inside each infected cell. Macrophages eventually succumb to the infection, releasing tachyzoites into the surrounding tissues. Cells take up freed parasites adjacent to the release site (e.g., glial

cells, astrocytes, hepatocytes, neutrophils, cardiac muscle). *T. gondii* undergoes another round of replication until protective immune responses are elicited. As a result, extensive tissue damage can be incurred, often accompanied by a constellation of clinical signs and symptoms. An effective immune response, mediated by interleukin-12 and interferon- γ and involving monocytes, dendritic cells, neutrophils, T cells, and natural killer cells, limits the rate of parasite division.²⁶ Antibodies are thought not to play a role in controlling this phase of the infection.

Protective immunity does not result in the elimination of the parasite. Instead, in response to host defense mechanisms, tachyzoites are forced to differentiate into a second asexual stage known as the bradyzoite. This form divides by endodyogeny and endopolygeny, then organizes into a tissue cyst.²⁷ Interferon- γ -dependent, NO-mediated effector mechanisms maintain this state of latency by eliminating any parasites emerg-

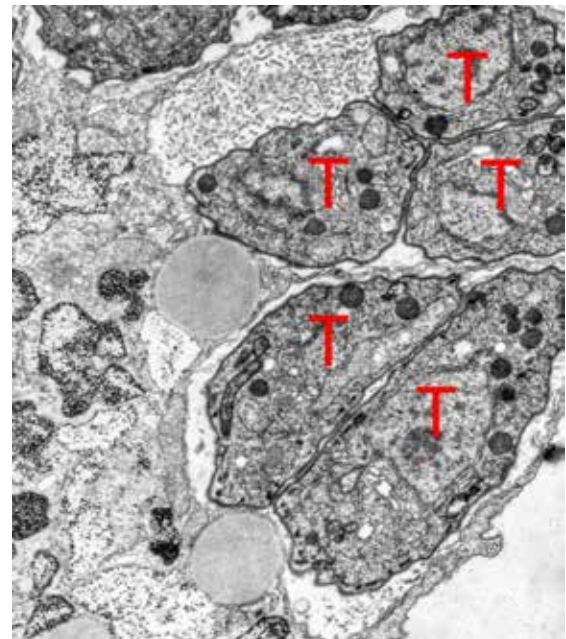


Figure 11.4. Transmission EM of a portion of infected macrophage. Note numerous tachyzoites (T). All parasites are alive; thus, the fusion of lysosomes with the parasitophorous vacuole is inhibited. Courtesy T. Jones.

Table 11.1. Congenital toxoplasmosis following maternal infection during first and second trimester*

Infective Status	Incidence
Not Infected	73%
Subclinical Infection	13%
Mild Infection	7%
Severe Infection	6%

*From Desmonts and Couvier, NEJM 290:1110, 1974

ing from the cysts.²⁸ Bradyzoites lie dormant in the tissues as long as host defenses remain active. Although all tissues can harbor tissue cysts, the brain, kidney, heart, and liver are favored sites for the long-term survival of the tissue cyst.²⁹

In addition to ingesting oocysts, intermediate and paratenic hosts can be infected by ingesting tissue cysts in the flesh of another intermediate host species. This route of transmission is most common among carnivores and scavengers. This is often a means of acquisition for humans who tend to ingest insufficiently cooked meat. Lamb, beef, and pork are the most common meats implicated in transmission worldwide.³⁰⁻³² The suggested internal temperature of 145 °F or 63 °C for beef cooking to kill *T. gondii* is the final cooked temperature of a medium-rare steak.

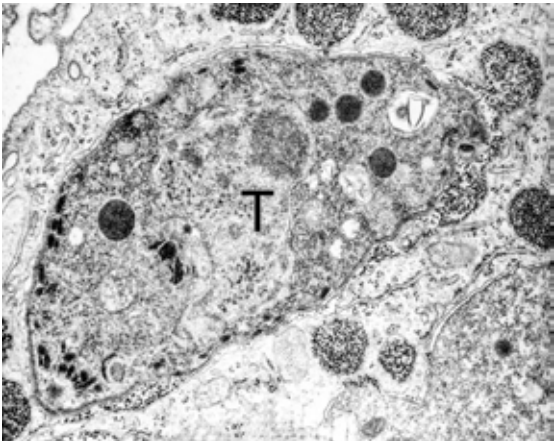


Figure 11.5. Transmission EM of a portion of macrophage that ingested a heat-killed tachyzoite. Note fusion of lysosomes with the parasitophorous vacuole (see top right fusion event. T = tachyzoite, Courtesy T. Jones.

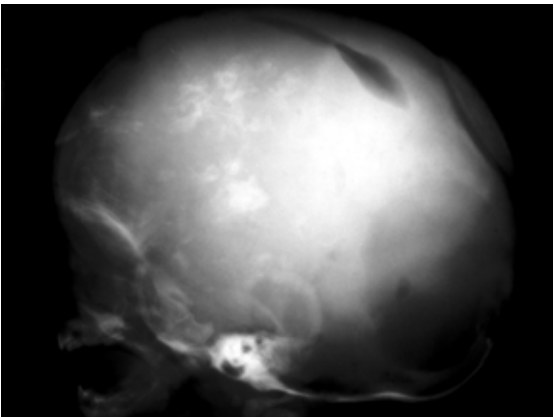


Figure 11.6. X-ray of the skull of an infant born with congenital toxoplasmosis. Infection was acquired most likely during the first or second trimester. Note calcifications.

Congenital transmission may occur from an infected mother to the fetus when tachyzoites cross the placenta. The role of specific antibodies in limiting infection to the mother and not the fetus has yet to be defined. Interferon- γ and CD8⁺ T cells appear necessary to prevent congenital infection in mouse models.³³

Cellular and Molecular Pathogenesis

Since *Toxoplasma* infection is not restricted by cell type, entry does not depend upon tissue-specific receptor molecules. Nevertheless, The process is complex and involves the coordinated, sequential deployment of specialized subcellular organelles: micronemes, rhoptries (lysosome-like granules), dense granules, and the glideosome.^{34, 35} Due to these organelles' biological activities unleashed upon the host cell, the tachyzoite can assume its intracellular life without hindrance from host defense mechanisms related to phagocytosis. *T. gondii* employs an entry scheme called a 'kiss and spit' approach, whereby it sprays virulence factors onto potential host cells and into already infected host cells.³⁶ Rhoptries, located at the apical end of the tachyzoite, and micronemes secrete adhesin-like molecules.^{35, 37-40} Parasite-specific secreted

Toxoplasma gondii

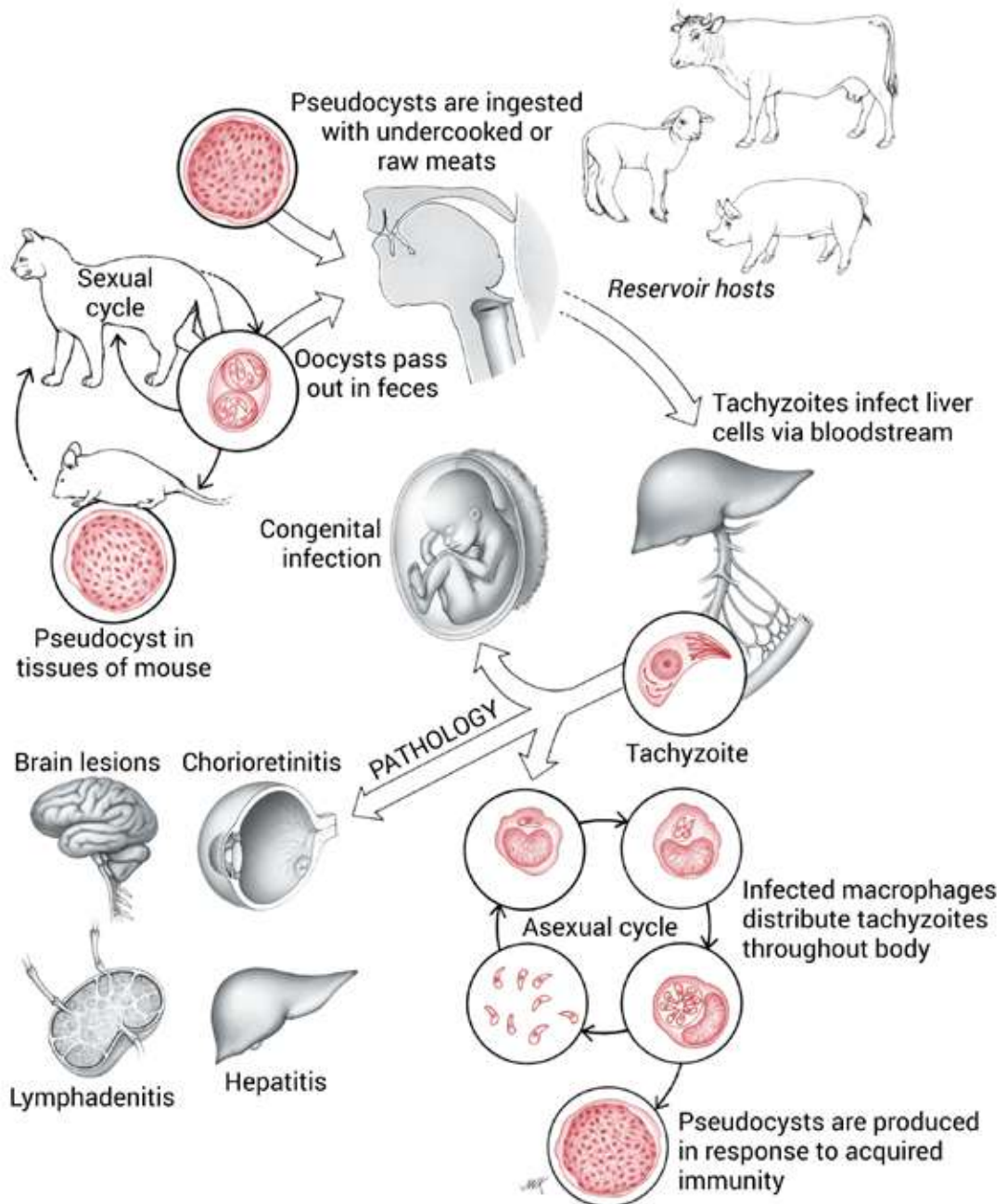


Table 11.2. Differential diagnosis of lymphadenopathy

Disorder	Toxoplasmosis	Inf. Mono	Lymphoma
Lymphadenopathy without other symptoms	+++	+	+++
Pharyngitis	+	+++	+
Monocytosis, eosinophilia	+++	+	+++
Atypical lymphocytes	+	++++	+++
Anemia	0	+	+++
Positive heterophil	0	++++	0
Altered liver function	0	++++	++
Hilar lymphadenopathy	+	+	+++
Lymph node pathology	Reticulum cells	Germinal cells	Bizarre cells

serine and cysteine proteases are required to engineer the parasitophorous vacuole where the tachyzoite lives and reproduces.^{37, 41, 42} These secreted serine and cysteine proteases are released, enabling the parasite to deform the target cell's cell membrane and remodel the vacuole's inner membrane. Inhibition of these two proteases prevents *T. gondii* from entering the cell.^{37, 41} The cDNA-encoding proteins from these organelles have been cloned and sequenced, and their amino acid sequences deduced.⁴³⁻⁴⁵ The Myr1 protein appears to be a key factor enabling the transit of molecules from the parasitophorous vacuole and is responsible for the organisms' virulence.⁴⁶

The parasite affects the arrangement of host cell organelles, including the mitochondria, which aggregate around the parasitophorous vacuole.⁴⁷ Division depends upon the ability

of the parasite to inhibit lysosomal fusion and inhibition of acidification of the parasitophorous vacuole.⁴⁸

Congenital toxoplasmosis is characterized by lesions of the CNS, which lead to various states of clinical disease.⁴⁹ Inflammatory lesions become necrotic and eventually calcify. Chorioretinitis is frequently associated with congenital toxoplasmosis.⁵⁰ The retina is inflamed and becomes necrotic, and the pigmented layer becomes disrupted by the infiltration of inflammatory cells. Eventually, granulation tissue forms and invades the vitreous humor. Brain tissue calcification (Fig. 11.6) is common when the fetus acquires infection during the first trimester. Hydrocephalus may result. Learning deficits in children who became infected in the first or second trimesters have been documented but are less common for those whose infec-

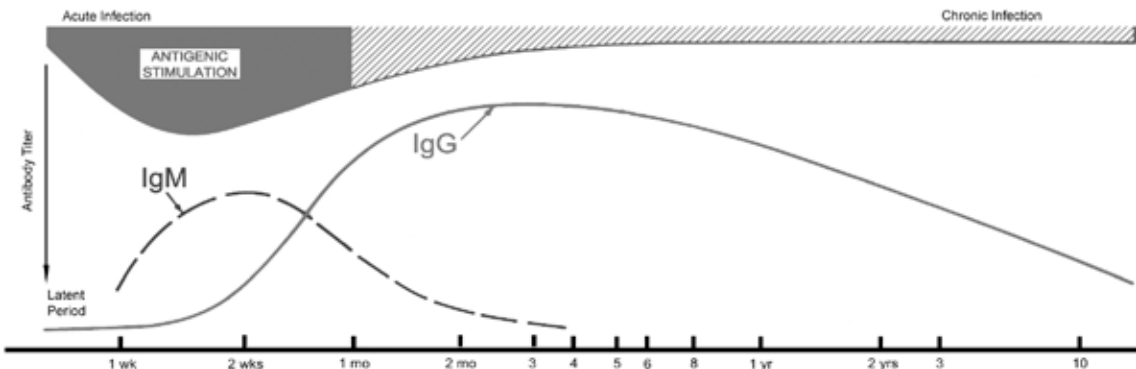


Figure 11.7. Relationship between antigenic stimulation, antibody production, and stages of infection (latent period, acute, and chronic infection). Redrawn after J. Remington.

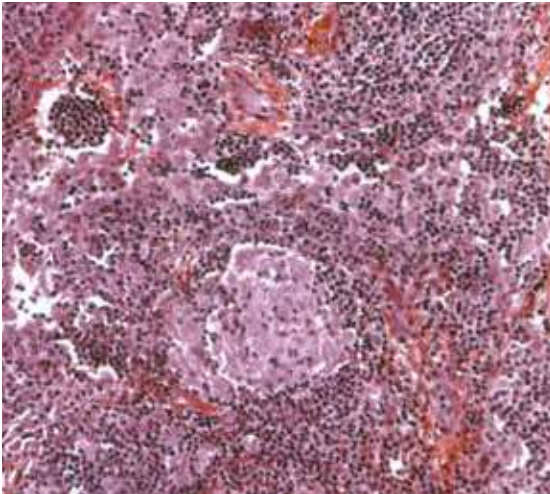


Figure 11.8. Histologic section of lymph node positive for macrophages infected with *T. gondii*. These cells are referred to as Piringa-Kuchenka cells.

tion occurred in the third trimester.

In adult-acquired toxoplasmosis, lesions are less intense, giving rise to foci of inflammation around tachyzoites in muscle and other tissues, such as the spleen, liver, and lymph nodes. Interstitial pneumonitis may also accompany infection.^{51, 52} Adult patients with AIDS who harbor latent infection with *T. gondii* suffer the most from reactivation of the infection.⁵³ This is mainly because HIV down-regulates interleukin-12 production and reduces the number of parasite-specific CD4⁺ and CD8⁺ T cells,⁵⁴⁻⁵⁶ dramatically reducing the interferon- γ dependent inhibition of parasite multiplication. Bradyzoites resume replication within tissue cysts and eventually rupture into the tissues, initiating infection in neighboring cells. *Toxoplasma* encephalitis results when reactivation occurs in the brain.⁵⁷ Reactivation can also occur in the lung, gastrointestinal (GI) tract, heart, eye, and liver. However, with the introduction of effective chemoprophylaxis for toxoplasmosis and HAART for HIV, clinical toxoplasmosis has been markedly reduced in recent years, even in resource-limited parts of the world.⁵⁸

Clinical Disease

There are three main manifestations of toxoplasmosis: congenital toxoplasmosis, acquired acute toxoplasmosis in the immunocompetent individual, and toxoplasmosis in the immunocompromised patient.^{59, 60}

Congenital Toxoplasmosis

Congenital infection varies from asymptomatic to severe damage to the CNS and still-birth (Table 11.1).⁶¹ Transmission typically occurs when a woman acquires a primary infection while pregnant. Transmission to the fetus is lowest when acute infection occurs during the first trimester, with only ~6% of fetuses acquiring infection. The percentage rises with gestational age of the fetus to over 70% if infection is acquired late in the third trimester.⁶² Fetal damage is most severe when infection occurs early in the pregnancy.⁶² Infection later in pregnancy constitutes most congenital toxoplasmosis, with most affected children asymptomatic. They may show infection's less severe pathological consequences several months to years later. The classic triad of chorioretinitis, hydrocephalus, and intracranial calcifications is present in less than 10% of cases.⁶³ Congenital toxoplasmosis can present as a subclinical infection, severe neonatal disease, disease in the first few months of life, or later as a relapse. Relapses often occur as ocular manifestations (e.g., chorioretinitis) when congenital infection goes unnoticed.⁶⁴ Of the infants that acquire infection *in utero*, about 15% have severe clinical manifestations.⁴⁹ Chorioretinitis leading to blindness, cerebral calcifications, and learning disabilities are the most frequent consequences.⁶⁵ Severely affected infants may have hepatosplenomegaly, liver failure, thrombocytopenia, convulsions, and hydrocephalus.⁶⁶ Without treatment, new lesions may continue to develop, increasing the likelihood of severe CNS impairment.⁶⁷

Acquired Acute Toxoplasmosis in the Immunocompetent Patient

It is estimated that 80–90% of acquired infections are asymptomatic. Those that are clinically apparent usually present as a mild self-limited disease. Symptomatic acute toxoplasmosis in the immunocompetent individual is often characterized by generalized lymphadenopathy, with predominant enlargement of the cervical nodes, sometimes associated with a low-grade fever (Table 11.2). The disease presents as an Epstein-Barr virus-negative mono-like illness. Rarely, adult-acquired toxoplasmosis is severe, involving major vital organs and systems. These patients may suffer myocarditis and encephalitis.^{68, 69} Chorioretinitis in acquired toxoplasmosis is rare, but *T. gondii* is one of the most common pathogens to cause posterior uveitis in immunocompetent hosts.

Toxoplasmosis in the Immunocompromised Patient

Encephalitis due to *T. gondii* is one of the causes of CNS disease in patients with AIDS.^{57, 70, 71} Toxoplasmosis in this setting is almost uniformly due to the reactivation of latent *T. gondii* acquired previously. HIV-infected patients at significant risk are those with CD4⁺ T cell counts <100 cells/μl with serological evidence of prior infection.⁷² Brain imaging usually reveals multiple ring-enhancing lesions. There can be extracerebral manifestations of toxoplasmosis in the immunocompromised patient. The lung is the next most common organ involved in reactivated toxoplasmosis, often manifesting as interstitial pneumonitis.^{51, 52} The GI tract, liver, and heart may also be affected. Cutaneous toxoplasmosis can present as a prominent macular and papular rash on the palms and soles.⁷³ An unusual form of acquired toxoplasmosis has been described in seronegative recipients of organ transplants from *T. gondii*-infected donors. Heart transplant recipients are particularly at risk and can develop myocarditis

or disseminated infection.⁷⁴ Severe recrudescent toxoplasmosis has also been described in patients undergoing immune suppression during bone marrow transplantation.⁷⁵

Diagnosis (see Clinical Appendix)

Definitive diagnosis is made by demonstrating the organism in histological sections or using PCR tests.^{76–79} PCR tests are instrumental in identifying organisms in ocular, amniotic, or CSF fluids.⁸⁰ Identifying tachyzoites in tissue sections without using antibody-based staining methods is challenging, even for a seasoned histopathologist. Indirect evidence of infection includes applying a wide variety of commercially available serological tests of several modalities (Fig. 11.7).⁸¹ These tests depend on the identification of specific immunoglobulins, IgG, and IgM. Various laboratory-based methods take advantage of these host responses to accurately diagnose active and inactive infection.

In most infections among otherwise healthy adults, IgM antibodies appear within five days to two weeks after infection and usually reach titers of 1:80 or more during the first 2–3 months after infection. They return to normal levels shortly after that. IgG antibody titers rise 2–3 weeks after infection and usually achieve levels above 1:1024. Specific IgG antibodies are detectable for life. Significant rises in titers of IgG antibodies between acute and convalescent serum specimens are highly correlated with acute infection. A single elevated IgM titer early in the infection can also support the diagnosis in the proper clinical context.

In some areas of the world, women at increased risk are recommended to undergo screening during pregnancy.⁸² Sero-conversion during pregnancy can be documented early in infection and closely monitored. Congenital infection can be confirmed when

specific IgM antibodies are detected in the infant's serum. About 25% of newborns with congenital toxoplasmosis have IgM antibodies. In contrast, infants with IgG antibodies pose a problem to the clinician in deciding whether or not the fetus was ever infected or whether the IgG represents a passive transfer of maternal antibody. Careful clinical and serological follow-up of the child is indicated to determine whether intrauterine infection has occurred.

Diagnosing toxoplasmosis in patients with AIDS often involves making a presumptive diagnosis and then a therapeutic trial.⁸³ The triad of a compatible clinical syndrome, a positive toxoplasma IgG, and typical brain imaging demonstrating multiple ring-enhancing lesions is associated with greater than a 90% positive predictive value for the diagnosis of CNS toxoplasmosis in an HIV-infected patient with a CD4⁺ T cell count <100 cells/ μ l.⁷² Histologic examination of lymph node tissue (Fig. 11.8) obtained at biopsy may show abnormal histiocytes, but this evidence is not pathognomonic for toxoplasmosis.

Treatment (see Clinical Appendix)

Mild infection in the immunocompetent patient does not require therapy unless the patient is pregnant and being treated to prevent fetus infection. In most cases, pyrimethamine and sulfadiazine are the drugs of choice.⁸⁴ For several reasons, alternative regimens are often necessary. If pyrimethamine is unavailable due to supply issues, cost, or intolerance, patients can be treated with trimethoprim-sulfamethoxazole or atovaquone.⁸⁵ In patients unable to tolerate sulfadiazine, alternative regimens containing atovaquone or azithromycin may be utilized.⁸⁶ The combination of pyrimethamine and sulfadiazine is teratogenic in animals, and there is concern regarding its use in pregnancy, particularly

during early trimesters.

Consequently, women who become acutely infected during pregnancy are often treated with the macrolide antibiotic spiramycin.⁸⁷ The ability of this agent to concentrate in the placenta is thought to help prevent transmission to the fetus.^{88, 89} Some physicians will treat women who acquire an infection with pyrimethamine and sulfadiazine during later trimesters. Still, in some settings, women will choose to terminate their pregnancies, as it is unclear whether initiation of treatment after the development of intracranial calcifications has any benefit.⁹⁰

Prevention and Control

Eating only well-cooked meats and avoiding the accidental ingestion of cat feces contaminated with toxoplasma oocysts can prevent most infections. These preventive measures often fail because of cultural or individual cuisine preferences. In France, where infection is over 85% among those over 50, the most common source of infection is raw meat served as "steak" tartar. Many other cultures also have numerous recipes in which undercooked or raw meat is a main ingredient. Several Alaskan native peoples still eat some of their meat raw and are, therefore, at the mercy of the pathogens lurking inside each carcass.⁹¹ In Ethiopia, raw meat with melted butter, kitfo, is a popular but risky dietary choice.⁹² Prevention of infection from oocysts requires care when handling cat feces, especially when cleaning litter boxes.⁹³ Pregnant women diminish their risk of infection by avoiding these activities. Rarely toxoplasmosis is acquired from inhaling dust or drinking water contaminated with oocysts.⁹⁴ Vaccines potentially offer another set of strategies for controlling this infection. There is a veterinary vaccine based on an attenuated pathogen, and research on a human vaccine is ongoing.⁹⁵⁻⁹⁷

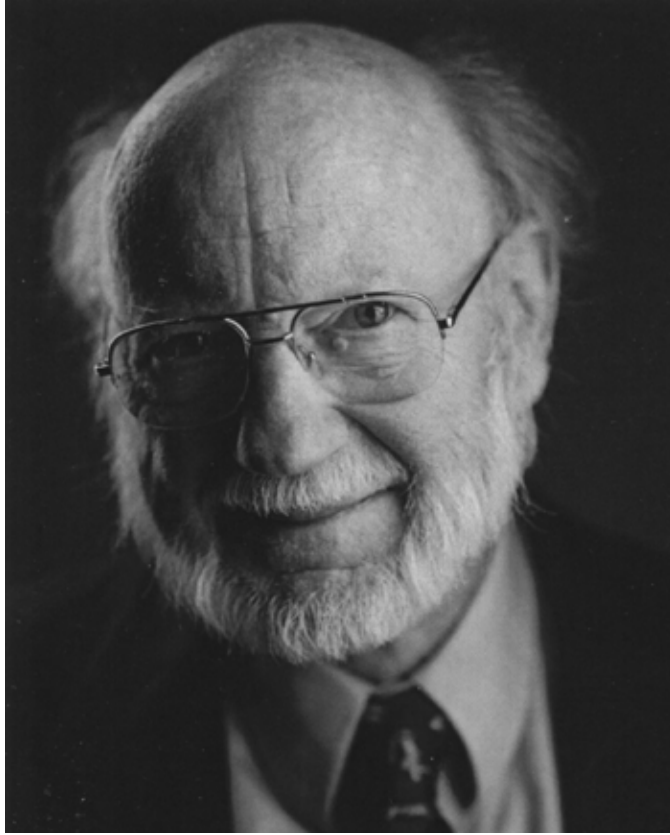
References

1. Dubey, J. P., A review of toxoplasmosis in wild birds. *Vet Parasitol* **2002**, *106* (2), 121-53.
2. Pappas, G., et al., Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. *Int J Parasitol* **2009**, *39* (12), 1385-94.
3. Miller, M. A., et al., An unusual genotype of *Toxoplasma gondii* is common in California sea otters (*Enhydra lutris nereis*) and is a cause of mortality. *International journal for parasitology* **2004**, *34* (3), 275-84.
4. Kim, K.; Weiss, L. M., *Toxoplasma gondii*: the model apicomplexan. *International journal for parasitology* **2004**, *34* (3), 423-32.
5. Gubbels, M.-J.; Striepen, B., Studying the cell biology of apicomplexan parasites using fluorescent proteins. *Microscopy and microanalysis : the official journal of Microscopy Society of America, Microbeam Analysis Society, Microscopical Society of Canada* **2004**, *10* (5), 568-79.
6. Boothroyd, J. C., *Toxoplasma gondii*: 25 years and 25 major advances for the field. *Int J Parasitol* **2009**, *39* (8), 935-46.
7. Kissinger, J. C., et al., ToxoDB: accessing the *Toxoplasma gondii* genome. *Nucleic acids research* **2003**, *31* (1), 234-6.
8. Khan, A., et al., Common inheritance of chromosome Ia associated with clonal expansion of *Toxoplasma gondii*. *Genome Res* **2006**, *16* (9), 1119-25.
9. Pozio, E., Foodborne and waterborne parasites. *Acta microbiologica Polonica* **2003**, *52 Suppl*, 83-96.
10. Dubey, J. P., Toxoplasmosis - a waterborne zoonosis. *Vet Parasitol* **2004**, *126* (1-2), 57-72.
11. Halonen, S. K.; Weiss, L. M., Toxoplasmosis. *Handb Clin Neurol* **2013**, *114*, 125-45.
12. Nicolle, C., et al., Sur une infection a coyotes de *Leishman* (ou organismes voisins) du gondi. *Seance Acad Sci* **1908**, *147*, 763-766.
13. Splendore, A., Un nuovo protozoa parassita dei conigli: incontrato nelle lesioni anatomiche d'ua maittia che ricorda in molti punti ii Kala-azar dell'uomo. *Rev Soc Sci Sao Paulo* **1908**.
14. Janku, J.; J., Pathogenesis and pathologic anatomy of the "congenital coloboma" of the maculalutea in an eye of normal size, with microscopic detection of parasites in the retina. *Phys* **1923**, *62*, 1021-1027.
15. Wolf, A., et al., Human toxoplasmosis: occurrence in infants as an encephalomyelitis verification by transmission to animals. *Science (New York, N.Y.)* **1939**, *89* (2306), 226-7.
16. Pinkerton, H.; Henderson, R. G., Adult toxoplasmosis: a previously unrecognized disease entity simulating the typhus-spotted fever group. *JAMA* **1941**, *116* 807-814.
17. Sabin, A. B., Toxoplasmic encephalitis in children. *JAMA* **1941**, *116* 801-807.
18. Frenkel, J. K., et al., *Toxoplasma gondii* in cats: fecal stages identified as coccidian oocysts. *Science (New York, N.Y.)* **1970**, *167* (3919), 893-6.
19. Hutchison, W. M., et al., Coccidian-like nature of *Toxoplasma gondii*. *Br Med J* **1970**, *1* (5689), 142-4.
20. Bresciani, K. D., et al., Experimental toxoplasmosis in pregnant bitches. *Vet Parasitol* **1999**, *86* (2), 143-5.
21. Tenter, A. M., et al., *Toxoplasma gondii*: from animals to humans. *Int J Parasitol* **2000**, *30* (12-13), 1217-58.
22. Bhopale, G. M., Development of a vaccine for toxoplasmosis: current status, *Microbes Infect. Microbes and infection* **2003**, *5* (5), 457-462.
23. Verma, R.; Khanna, P., Development of *Toxoplasma gondii* vaccine: A global challenge. *Hum Vaccin Immunother* **2013**, *9* (2), 291-3.
24. Jones, T. C.; Hirsch, J. G., The interaction between *Toxoplasma gondii* and mammalian cells. II. The absence of lysosomal fusion with phagocytic vacuoles containing living parasites. *The Journal of experimental medicine* **1972**, *136* (5), 1173-94.
25. Dlugonska, H.; J., Molecular modifications of host cells by *Toxoplasma gondii*. *Pol* **2004**, *53*, 45-

- 54.
26. Hunter, C. A.; Sibley, L. D., Modulation of innate immunity by *Toxoplasma gondii* virulence effectors. *Nat Rev Microbiol* **2012**, *10* (11), 766-78.
27. Dzierzinski, F., et al., Dynamics of *Toxoplasma gondii* differentiation. *Eukaryotic cell* **2004**, *3* (4), 992-1003.
28. Scharton-Kersten, T. M., et al., Inducible nitric oxide is essential for host control of persistent but not acute infection with the intracellular pathogen *Toxoplasma gondii*. *The Journal of experimental medicine* **1997**, *185* (7), 1261-73.
29. Dadimoghaddam, Y., et al., Tissue tropism and parasite burden of *Toxoplasma gondii* RH strain in experimentally infected mice. *Asian Pac J Trop Med* **2014**, *7* (7), 521-4.
30. Williams, R. H., et al., High levels of congenital transmission of *Toxoplasma gondii* in longitudinal and cross-sectional studies on sheep farms provides evidence of vertical transmission in ovine hosts. *Parasitology* **2005**, *130* (Pt 3), 301-7.
31. Dubey, J. P., et al., High prevalence of viable *Toxoplasma gondii* infection in market weight pigs from a farm in Massachusetts. *J Parasitol* **2002**, *88* (6), 1234-8.
32. Dubey, J. P., et al., Toxoplasmosis in black-faced kangaroos (*Macropus fuliginosus melanops*). *Vet Parasitol* **1988**, *30* (2), 97-105.
33. Abou-Bacar, A., et al., Role of gamma interferon and T cells in congenital *Toxoplasma* transmission. *Parasite immunology* **2004**, *26* (8-9), 315-8.
34. Carruthers, V. B.; Blackman, M. J., A new release on life: emerging concepts in proteolysis and parasite invasion. *Molecular microbiology* **2005**, *55* (6), 1617-30.
35. Keeley, A.; Soldati, D., The glideosome: a molecular machine powering motility and host-cell invasion by Apicomplexa. *Trends in cell biology* **2004**, *14* (10), 528-32.
36. Boothroyd, J. C.; Dubremetz, J. F., Kiss and spit: the dual roles of *Toxoplasma* rhoptries. *Nat Rev Microbiol* **2008**, *6* (1), 79-88.
37. Que, X., et al., The cathepsin B of *Toxoplasma gondii*, toxopain-1, is critical for parasite invasion and rhoptry protein processing. *The Journal of biological chemistry* **2002**, *277* (28), 25791-7.
38. Ngo, H. M., et al., Are rhoptries in Apicomplexan parasites secretory granules or secretory lysosomal granules? *Mol Microbiol*. **2004**, *52* 1531-41.
39. Carruthers, V. B., et al., Secretion of micronemal proteins is associated with *Toxoplasma* invasion of host cells. *Cellular microbiology* **1999**, *1* (3), 225-35.
40. Huynh, M.-H., et al., Rapid invasion of host cells by *Toxoplasma* requires secretion of the MIC2-M2AP adhesive protein complex. *The EMBO journal* **2003**, *22* (9), 2082-90.
41. Conseil, V., et al., Serine protease inhibitors block invasion of host cells by *Toxoplasma gondii*. *Antimicrobial agents and chemotherapy* **1999**, *43* (6), 1358-61.
42. Kim, K., Role of proteases in host cell invasion by *Toxoplasma gondii* and other Apicomplexa. *Acta tropica* **2004**, *91* (1), 69-81.
43. Ajioka, J. W., et al., Gene discovery by EST sequencing in *Toxoplasma gondii* reveals sequences restricted to the Apicomplexa. *Genome research* **1998**, *8* (1), 18-28.
44. Yahiaoui, B., et al., Isolation and characterization of a subtractive library enriched for developmentally regulated transcripts expressed during encystation of *Toxoplasma gondii*. *Molecular and biochemical parasitology* **1999**, *99* (2), 223-35.
45. Bradley, P. J., et al., A GFP-based motif-trap reveals a novel mechanism of targeting for the *Toxoplasma* ROP4 protein. *Molecular and biochemical parasitology* **2004**, *137* (1), 111-20.
46. Franco, M., et al., A Novel Secreted Protein, MYR1, Is Central to *Toxoplasma*'s Manipulation of Host Cells. *MBio* **2016**, *7* (1), e02231-15.
47. Lindsay, D. S., et al., Association of host cell mitochondria with developing *Toxoplasma gondii* tissue cysts. *American journal of veterinary research* **1993**, *54* (10), 1663-7.
48. Shaw, M. K., et al., Acidic compartments and rhoptry formation in *Toxoplasma gondii*. *Parasitology* **1998**, *117* (Pt 5), 435-43.
49. Remington, J. S., et al., Toxoplasmosis. In: Infectious Diseases of the Fetus and Newborn Infant. 1995.
50. Mets, M. B., et al., Eye manifestations of congenital toxoplasmosis. *American journal of*

- ophthalmology* **1997**, 123 (1), 1-16.
51. Campagna, A. C., Pulmonary toxoplasmosis. *Seminars in respiratory infections* **1997**, 12 (2), 98-105.
 52. Mariuz, P., et al., *Toxoplasma pneumonia*. *Seminars in respiratory infections* **1997**, 12 (1), 40-3.
 53. Kaplan, J. E.; V., H. I., Diagnosis, treatment, and prevention of selected common HIV-related opportunistic infections in the Caribbean region. *Top* **2005**, 12, 136-41.
 54. Vanham, G., et al., Decreased CD40 ligand induction in CD4 T cells and dysregulated IL-12 production during HIV infection. *Clinical and experimental immunology* **1999**, 117 (2), 335-42.
 55. Sartori, A., et al., Interleukin-12: an immunoregulatory cytokine produced by B cells and antigen-presenting cells. *Methods (San Diego, Calif.)* **1997**, 11 (1), 116-27.
 56. Alonso, K., et al., Cytokine patterns in adults with AIDS. *Immunological investigations* **1997**, 26 (3), 341-50.
 57. Luft, B. J.; Remington, J. S., Toxoplasmic encephalitis in AIDS. *Clin Inf Dis* **1992**, 15 (2), 211-22.
 58. Hari, K. R., et al., Reduced risk of toxoplasma encephalitis in HIV-infected patients--a prospective study from Gauteng, South Africa. *Int J STD AIDS* **2007**, 18 (8), 555-8.
 59. Hill, D.; Dubey, J. P., *Toxoplasma gondii*: transmission, diagnosis and prevention. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **2002**, 8 (10), 634-40.
 60. Montoya, J. G.; Liesenfeld, O., Toxoplasmosis. *Lancet (London, England)* **2004**, 363 (9425), 1965-76.
 61. Desmont, G.; Couvreur, J., Congenital toxoplasmosis: a prospective study of 378 pregnancies. *N Engl J Med* **1974**, 290 S, 1110-1116.
 62. Montoya, J. G.; Remington, J. S., Management of *Toxoplasma gondii* infection during pregnancy. *Clin Infect Dis* **2008**, 47 (4), 554-66.
 63. Tamma, P., Toxoplasmosis. *Pediatr Rev* **2007**, 28 (12), 470-1.
 64. Wilson, C. B., et al., Development of adverse sequelae in children born with subclinical congenital *Toxoplasma* infection. *Pediatrics* **1980**, 66 (5), 767-74.
 65. Kravetz, J. D.; Federman, D. G., Toxoplasmosis in pregnancy. *The American journal of medicine* **2005**, 118 (3), 212-6.
 66. Koppe, J. G., et al., Results of 20-year follow-up of congenital toxoplasmosis. *Lancet (London, England)* **1986**, 1 (8475), 254-6.
 67. Bhopale, G. M., Pathogenesis of toxoplasmosis. *Comparative immunology, microbiology and infectious diseases* **2003**, 26 (4), 213-22.
 68. Kirchhoff, L. V., et al., Parasitic diseases of the heart. *Frontiers in bioscience* **2004**, 9, 706-23.
 69. Wilson, E. H.; Hunter, C. A., The role of astrocytes in the immunopathogenesis of toxoplasmic encephalitis. *International journal for parasitology* **2004**, 34 (5), 543-8.
 70. Nath, A.; Sinai, A. P., Cerebral Toxoplasmosis. *Current treatment options in neurology* **2003**, 5 (1), 3-12.
 71. Leyva, W. H.; Santa Cruz, D. J., Cutaneous toxoplasmosis. *Journal of the American Academy of Dermatology* **1986**, 14 (4), 600-5.
 72. Belanger, F., et al., Incidence and risk factors of toxoplasmosis in a cohort of human immunodeficiency virus-infected patients: 1988-1995. HEMOCO and SEROCO Study Groups. *Clin Infect Dis* **1999**, 28 (3), 575-81.
 73. Janitschke, K., et al., Diagnostic value of tests for *Toxoplasma gondii*-specific antibodies in patients undergoing bone marrow transplantation. *Clinical laboratory* **2003**, 49 (5-6), 239-42.
 74. Wreghitt, T. G., et al., Toxoplasmosis in heart and heart and lung transplant recipients. *J Clin Pathol* **1989**, 42 (2), 194-9.
 75. Mele, A., et al., Toxoplasmosis in bone marrow transplantation: a report of two cases and systematic review of the literature. *Bone Marrow Transplant* **2002**, 29 (8), 691-8.
 76. Nimri, L., et al., Detection of *Toxoplasma gondii* DNA and specific antibodies in high-risk pregnant women. *The American journal of tropical medicine and hygiene* **2004**, 71 (6), 831-5.
 77. Danise, A., et al., Use of polymerase chain reaction assays of aqueous humor in the differential diagnosis of retinitis in patients infected with human immunodeficiency virus. *Clin Infect Dis* **1997**, 24 (6), 1100-6.

78. Bergstrom, T., et al., Congenital *Toxoplasma gondii* infection diagnosed by PCR amplification of peripheral mononuclear blood cells from a child and mother. *Scand Dis* **1998**, 30, 202-4.
79. Wilson, M., et al., Evaluation of six commercial kits for detection of human immunoglobulin M antibodies to *Toxoplasma gondii*. The FDA Toxoplasmosis Ad Hoc Working Group. *Journal of clinical microbiology* **1997**, 35 (12), 3112-5.
80. Costa, J. G., et al., Real-time PCR as a prognostic tool for human congenital toxoplasmosis. *J Clin Microbiol* **2013**, 51 (8), 2766-8.
81. Petersen, E., et al., European multicenter study of the LIAISON automated diagnostic system for determination of *Toxoplasma gondii*-specific immunoglobulin G (IgG) and IgM and the IgG avidity index. *Journal of clinical microbiology* **2005**, 43 (4), 1570-4.
82. Paquet, C., et al., Toxoplasmosis in pregnancy: prevention, screening, and treatment. *J Obstet Gynaecol Can* **2013**, 35 (1), 78-81.
83. Malla, N., et al., Antigenaemia and antibody response to *Toxoplasma gondii* in human immunodeficiency virus-infected patients. *British journal of biomedical science* **2005**, 62 (1), 19-23.
84. Petersen, E.; Schmidt, D. R., Sulfadiazine and pyrimethamine in the postnatal treatment of congenital toxoplasmosis: what are the options? *Expert review of anti-infective therapy* **2003**, 1 (1), 175-82.
85. Torre, D., et al., Randomized trial of trimethoprim-sulfamethoxazole versus pyrimethamine-sulfadiazine for therapy of toxoplasmic encephalitis in patients with AIDS. Italian Collaborative Study Group. *Antimicrob Agents Chemother* **1998**, 42 (6), 1346-9.
86. Ramos, M. E. H.; Villaraza, S. G., The use of azithromycin and pyrimethamine for treatment of cerebral toxoplasmosis in human immunodeficiency virus-infected patients: a systematic review. *Encephalitis* **2023**, 3 (2), 64-70.
87. Montoya, J. G., et al., A fresh look at the role of spiramycin in preventing a neglected disease: meta-analyses of observational studies. *Eur J Med Res* **2021**, 26 (1), 143.
88. Chang, H. R.; Pechere, J. C., *In vitro* effects of four macrolides (roxithromycin, spiramycin, azithromycin [CP-62,993], and A-56268) on *Toxoplasma gondii*. *Antimicrob Agents Chemother* **1988**, 32 (4), 524-9.
89. Couvreur, J., et al., Prophylaxis of congenital toxoplasmosis. Effects of spiramycin on placental infection. *J Antimicrob Chemother* **1988**, 22 Suppl B, 193-200.
90. Cortina-Borja, M., et al., Prenatal treatment for serious neurological sequelae of congenital toxoplasmosis: an observational prospective cohort study. *PLoS Med* **2010**, 7 (10).
91. Boyer, K. M., et al., Risk factors for *Toxoplasma gondii* infection in mothers of infants with congenital toxoplasmosis: Implications for prenatal management and screening. *American journal of obstetrics and gynecology* **2005**, 192 (2), 564-71.
92. Muleta, D.; Ashenafi, M., Salmonella, Shigella and growth potential of other food-borne pathogens in Ethiopian street vended foods. *East Afr Med J* **2001**, 78 (11), 576-80.
93. Teutsch, S. M., et al., Epidemic toxoplasmosis associated with infected cats. *N Engl J Med* **1979**, 300 (13), 695-9.
94. Isaac-Renton, J., et al., Detection of *Toxoplasma gondii* oocysts in drinking water. *Applied and environmental microbiology* **1998**, 64 (6), 2278-80.
95. Beghetto, E., et al., A combination of antigenic regions of *Toxoplasma gondii* microneme proteins induces protective immunity against oral infection with parasite cysts. *The Journal of infectious diseases* **2005**, 191 (4), 637-45.
96. Chu, K. B.; Quan, F. S., Advances in *Toxoplasma gondii* Vaccines: Current Strategies and Challenges for Vaccine Development. *Vaccines (Basel)* **2021**, 9 (5).
97. Hasan, T.; Nishikawa, Y., Advances in vaccine development and the immune response against toxoplasmosis in sheep and goats. *Front Vet Sci* **2022**, 9, 951584.



William Cecil Campbell, Ph.D. (born 1930)

For their seminal work, Satoshi Omura and Campbell shared the 2015 Nobel Prize in Physiology or Medicine, for therapies that revolutionized the treatment of certain parasitic diseases. Together with Satoshi Omura, Campbell discovered a fermentation product from a soil-dwelling bacteria *Streptomyces avermilitis*, avermectin, which proved to have remarkable anti-helminthic properties. A derivative, ivermectin, is commercially available and is the drug responsible for eliminating the filarial worm *Onchocerca volvulus* (responsible for river blindness) from most of West Africa. This work was supported in part by the Carter Center, Merck, and the cooperating countries in West Africa. Other helminthic infections (both human and animal) also are treatable with ivermectin.

12. *Entamoeba histolytica*

(Schaudinn 1903)

Pronunciation: \en-tə-'mē-bə\\
his'təlīt'ikə\\

Introduction

Entamoeba histolytica (\ent-a-MEE-ba\\HIS-tow-lit-i-ka\\) is the causative agent of amoebic dysentery in humans.^{1,2} While most amoebic infections are due to *E. histolytica*, other species of amoebae are free-living, and other members of this genus (e.g., *Entamoeba dispar* and *Entamoeba moshkovskii* (\ent-a-MEE-ba mosh-KOV-ski-ee\\), can inhabit the human large intestine without causing disease. It is suggested that some strains of *Entamoeba dispar* can cause disease of both the intestines and the liver in humans.³ There is also the suggestion that *E. moshkovskii* can cause childhood diarrhea or that a different subspecies (*Entamoeba bangladeshi*) may cause disease.^{2,4,6}

E. histolytica is transmitted from person to person via the fecal-oral route, taking up residence in the wall of the large intestine.⁷
⁸ Protracted infection can progress from watery diarrhea to dysentery (bloody diarrhea), which may prove fatal if left untreated. In addition, *E. histolytica* can spread to extra-intestinal sites, causing severe disease.⁹ *E. histolytica* lives as a trophozoite in the host's tissues and as a resistant cyst in the outside environment. Sanitation programs that limit exposure to food and water-borne diarrheal disease agents effectively limit infection with *E. histolytica*. Some animals (non-human primates and domestic dogs) can become infected with *E. histolytica*, spreading the pathogen by coprophagia, but none serve as important reservoirs for human infection.¹⁰

E. dispar and *E. moshkovskii* are morphologically identical amoebae that can be misiden-

tified as *E. histolytica* during microscopic examination of fecal samples.¹¹ Commercially available stool antigen detection tests and NAATs are now widely available that are sensitive and specific, allowing for the detection of *E. histolytica* and discrimination between *E. histolytica* and nonpathogenic amoebae.^{12,13} Much is now known about the basic biology and clinical aspects of infection with *E. histolytica*, and the genome has been sequenced.¹⁴⁻¹⁶

Historical Information

Although reports of what appears to be amoebiasis can be found in the writings of Hippocrates (460–377 BCE) and the Old Testament, appreciation for its significance as a pathogen began when Fedor Losch, in 1875, described clinical features of infection with *E. histolytica* and reproduced some aspects of the disease in experimentally infected dogs.^{17,18} In 1891, William Councilman and Henri Lafleur described the main features of the intestinal pathogenesis caused by *E. histolytica*.¹⁹ A couple of years later, in 1893, Heinrich Quincke and Ernst Roos distinguished *E. histolytica* from *Entamoeba*

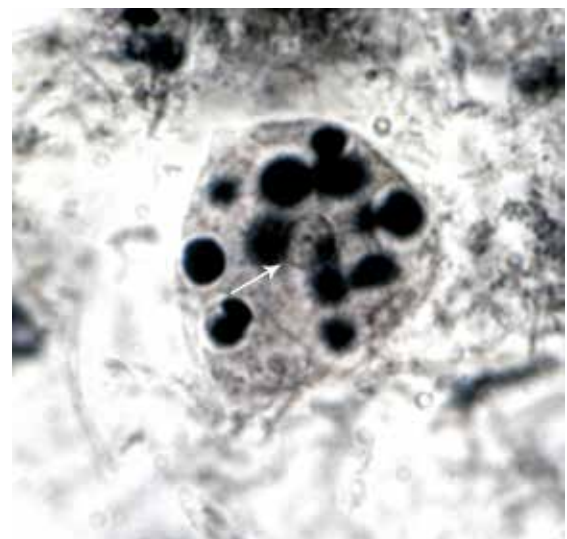


Figure 12.1. Trophozoite of *Entamoeba histolytica*. Note nucleus (arrow) and numerous ingested red cells. 35 μ m.

coli, a non-pathogenic amoeba acquired by the fecal-oral route and often found in the stool of asymptomatic individuals.²⁰ A decade later, in 1903, Fritz Schaudinn described the trophozoites and cysts of *E. histolytica*.²¹ Fritz Schaudinn died at the age of 35 of overwhelming amoebiasis, a tragic consequence of self-experimentation. In 1925, William Boeck was the first to culture *E. histolytica*, while Clifford Dobell, in 1928, fully elucidated its life cycle.²²⁻²⁴

Life Cycle

The trophozoite (Fig. 12.1) is a facultative anaerobe metabolizing glucose as its primary energy source.²⁵ The trophozoite measures 20–30 μm in diameter, and the cytoplasm contains a single nucleus with a centrally located nucleolus, often termed the karyosome. In addition, surface-soluble lysosomes and a remnant mitochondrion organelle called a crypton or mitosome are present.²⁶⁻²⁸ *Entamoeba* spp. evolved after the evolution of the mitochondrial-containing eukaryotes, and the mitosome is thought to be a remnant organelle resulting from organelle decay of

the mitochondria.^{29, 30} Evidence suggests that the mitosome no longer contains any DNA and likely lost its genome through reductive evolution.²⁹ Discovery of a calreticulin-like molecule of 51 kDa suggested the presence of endoplasmic reticulum (ER), and further work has now verified the presence of a continuous ER in *E. histolytica*.^{31, 32}

The cyst (Fig. 12.2) is smaller than the trophozoite (10–15 μm in diameter) and contains four typically round *E. histolytica* nuclei at full maturity. Each nucleus ultimately will give rise to an individual trophozoite. Immature cysts may have a single, smooth-ended chromatoidal bar, a crystalline-like condensation of ribosomes, and any number of nuclei up to four.

Ingestion of a single cyst is necessary to initiate infection, making this organism one of the most efficient pathogenic protozoa known to infect humans.³³ Each cyst undergoes excystation in the small intestine. Excystation is complex, involving actin cytoskeletal reorganization.^{34, 35} The cyst must receive specific environmental cues from the host, including sequential exposure to an acidic and then a basic pH environment, for the single quadri-nucleate cell to undergo cytokinesis, exit through the cyst wall, and enter the small

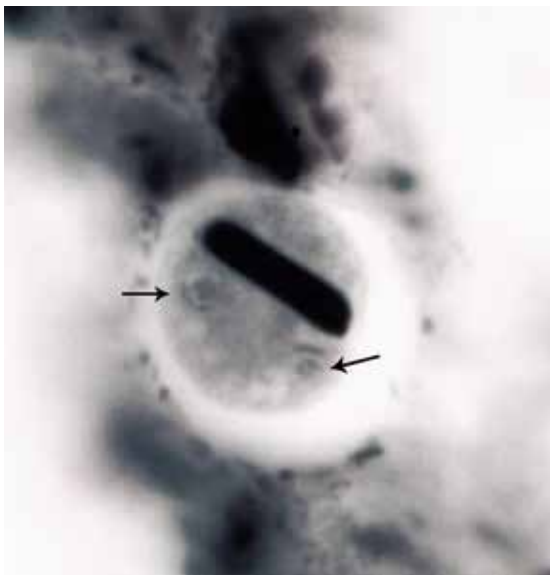


Figure 12.2. Cyst of *E. histolytica*. Two nuclei (arrows) and a smooth-ended chromatoidal bar can be seen. 15 μm .

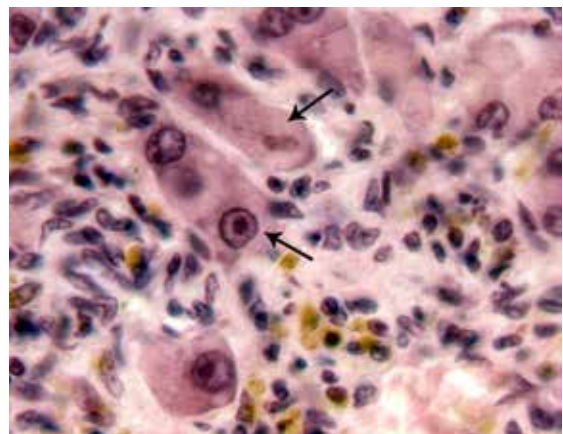
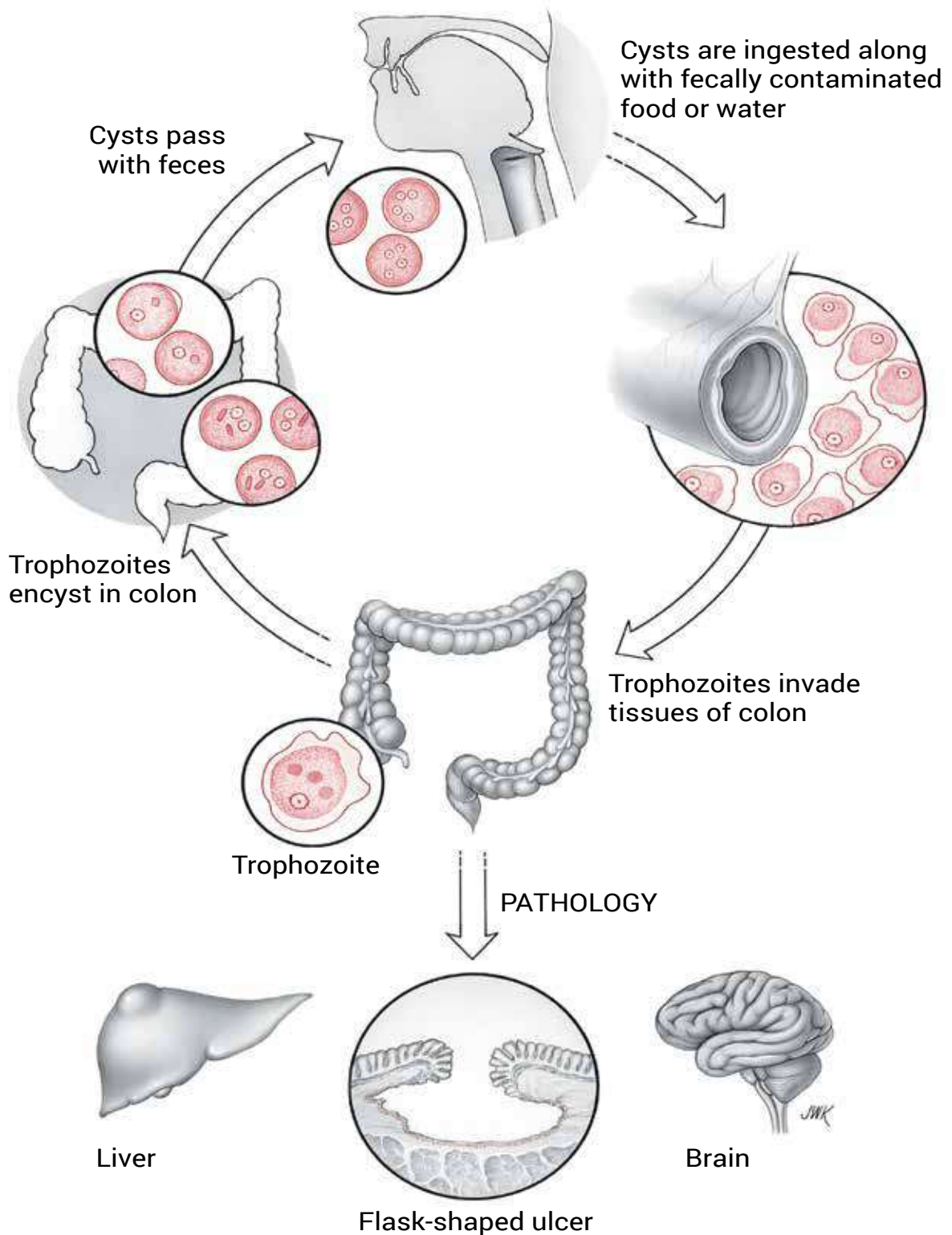


Figure 12.3. Trophozoites of *E. histolytica* in liver abscess (arrows). Note ingested host cells inside parasites.

Entamoeba histolytica



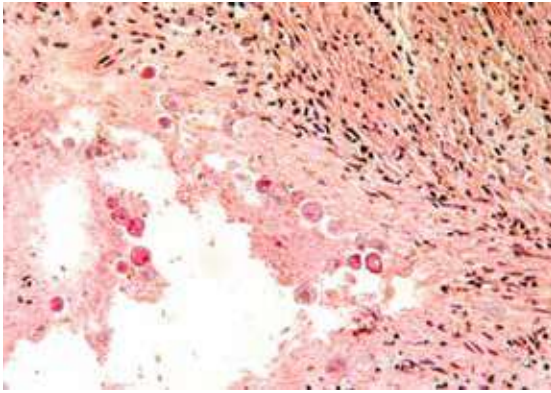


Figure 12.4. Low-magnification histologic section of amoebic ulcer in small intestine. Organisms can be seen at living margin of ulcer.

intestine. The four newly emerged trophozoites then divide, and the resulting eight parasites are carried by peristalsis to the large intestine. There is no sexual phase, and consequently, replication is clonal.⁹

In the large intestine, the trophozoite penetrates the perimucosal space and attaches to epithelial cells using lectin-galactose interactions.³⁶ This event is cytotoxic.³⁷ The amoeba engulfs and kills only living cells (Fig. 12.3).³⁶ Trophozoites divide by binary fission, occupying increasingly larger areas of tissue as they do so.²⁴ This activity eventually causes flask-shaped ulcers to develop (Fig. 12.4).

Hematogenous or lymphatic spread is possible, but this aspect does not play a role in the life cycle.

Some trophozoites, instead of dividing, encyst in the lumen of the ulcer. Encystation also involves actin cytoskeletal reorganization, likely triggered by utilizing the Gal/GalNAc-specific lectin.^{34, 35} Amoebic proteasome activity may also be necessary since treating cultures with lactacystin caused marked inhibition of cyst formation.^{38, 39} Although early *in vitro* growth required feeder cells to culture *E. histolytica*, it can now be cultured in cell-free media, with excellent results, particularly when a layer of mineral oil is overlaid to enhance the anaerobic conditions.⁴⁰ During gastrointestinal (GI) tract infection, cysts may continuously produce and exit the host in feces. Cysts can survive in warm, moist conditions for weeks without losing infectivity.

Cellular and Molecular Pathogenesis

There is a complex process underlying the pathogenesis of intra-intestinal and extra-intestinal amoebiasis.^{15, 16, 41-45} Amoebae must

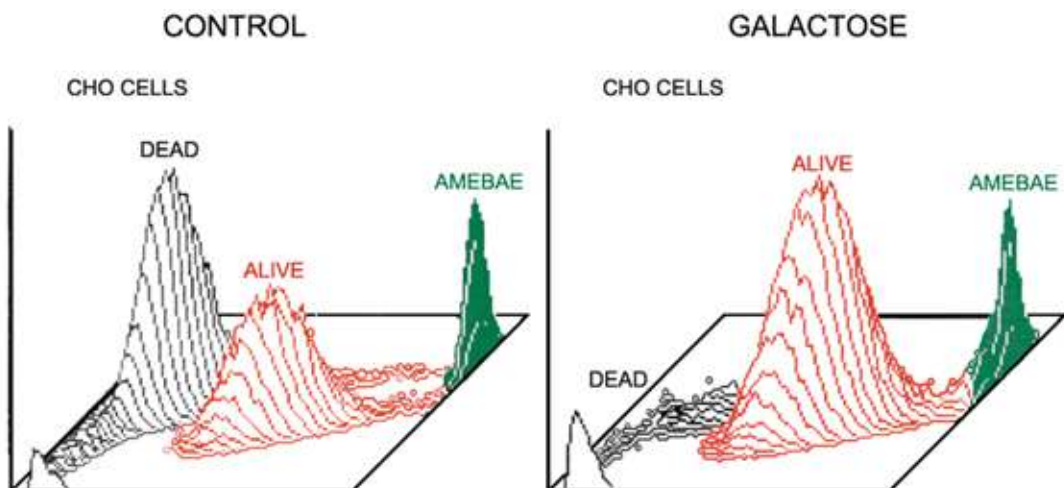


Figure 12.5. *In vitro* experiment showing that galactose-containing surface proteins are important for parasite cytotoxicity. Free galactose prevents attachment of amoebae to their target cells. CHO cells=Chinese hamster ovary cells. Redrawn after J. I. Ravdin.

attach to host tissues as a necessary prerequisite for parasite-mediated cytotoxicity. Attachment depends upon interactions between epithelial cell membrane-bound N-acetyl-glucosamine and N-acetyl-galactosamine and at least two surface lectin proteins. The genes for both parasite lectins have been cloned, and their cDNAs sequenced. One lectin is a 260 kDa protein, while the other is 220 kDa.⁴⁶ The heavy subunit of the 260 kDa lectin has a single transmembrane-spanning domain and a cytoplasmic domain related to β -2-integrins, which may also participate in the attachment process.^{47, 48} These surface lectins also facilitate the parasite's evasion of the complement membrane attack complex.⁴⁹

In vitro, *E. histolytica* can be inhibited from attaching to its target cells simply by adding free galactose to the medium (Fig. 12.5).⁵⁰ In this situation, cells and trophozoites coexist. Attachment leads to cell death, which, at least *in vitro*, is calcium-dependent.⁵¹ Several possible mechanisms for killing host cells have been proposed, all involving enzymes such as serine proteases.⁵² A recent finding has shown that trophozoites can “nibble” off a portion of their target cells just before killing them, a process termed trogocytosis.⁵³ Apparently, *E. histolytica* needs to sample its meal before fully committing to ingesting it. This pathogen turns out to be a fussy eater. Although inflammation and the release of cytokines may play a role in the tissue destruction associated with acute infection, a significant amount of tissue destruction is present in chronic ulcerations that is out of proportion to the degree of inflammation seen at that stage of the infection.⁵⁴

The trophozoite's surface membrane contains phospholipase A, neuraminidase, and a metallocollagenase.⁵⁵ It also secretes a minimum of four cysteine proteases.⁵⁶ These enzymes may also aid the parasite in moving

through the extracellular matrix. Attachment elicits the secretion of a pore-forming peptide that is biochemically related both in structure and function to saposins.^{57, 58} The pore-forming protein presumably plays a central role in lysing the host cell membrane. During attachment, the intracellular calcium levels of the target cell increase by 20-fold.⁵⁹ One intriguing finding is that the trophozoite may actually “lure in” new target cells, in this case, lymphocytes, to the site of infection by upregulating the chemotactic interleukin-8 in the surrounding colonic epithelial cells while simultaneously inhibiting the upregulation of other cytokines known to play a role in inflammation.^{60, 61}

Protective immune mechanisms are short-lived and depend on developing secretory IgA antibodies directed against parasite surface proteins involved in adherence to target cells, such as Gal/GalNAc lectin, a novel multifunctional virulence factor.⁶²⁻⁶⁶ In addition, cell-mediated killing of parasites can occur by induction of NO by the 220 kDa lectin, which up-regulates interferon- γ .^{67, 68} The importance of CD4⁺ T cells may be minimal as patients with HIV-1 infection and impaired T cell function do not seem to suffer dramatically increased rates of invasive disease.⁶⁹ In experimental infections, polyclonal and monoclonal antibodies effectively protect the host when directed against carbohydrate-binding lectins of the parasite, emphasizing the central role these parasite proteins play in disease pathogenesis.^{70, 71}

Clinical Disease

Intestinal amoebiasis

Most exposed individuals are asymptomatic, but a number will go on to experience clinical disease or become chronic carriers. Host factors such as genetic background, age, immune status, nutritional status, pregnancy, and co-morbidities may determine whether a

host becomes symptomatic and the severity and type of manifestation.

Those who are symptomatic may experience a wide range of clinical disease.^{43, 72} The most common manifestation is diarrhea, lasting more than a few days and sometimes for weeks, months, or years if not treated appropriately.^{43, 72} Although many patients do not report blood or excess mucous in their stools, almost all stools are heme-positive if tested.⁷³ Involvement of the entire bowel can be associated with colicky pain, flatulence, alteration in the pattern of bowel movements, bloody stools, and eventually dysentery, which must be distinguished clinically from ulcerative colitis. Although patients may be afebrile, many patients will report fever at clinical presentation.⁷³

Generalized abdominal tenderness, with particular accentuation in the iliac fossa, is frequently encountered on physical examination. Dysentery can either worsen, possibly resulting in a life-threatening situation, or resolve into a chronic state of ill health characterized by bouts of diarrhea, abdominal cramping, and abdominal discomfort. In chronic conditions, amoebomas (large granulomas of eosinophils, amoebae, and necrotic colonic tissue) are possible and pres-

ent as palpable masses. Amoebomas can be misdiagnosed as malignancies in the barium enema. If the disease progresses, the colon may become atonic and perforate at one or several points of ulceration (Fig. 12.6). If perforation occurs, symptoms and signs of peritonitis may develop. Acute colitis occurs more frequently in children.⁴³

The perforated, inflamed bowel may adhere to the abdominal wall and extend to the skin, causing cutaneous amoebiasis, which can progress rapidly.⁷⁴ This situation may also occur in the perianal area due to skin invasion by the trophozoites emerging from the rectum.

Extra-intestinal amoebiasis

Amoebae can erode the wall of the large intestine until the circulation of the submucosa is breached. In that case, parasites are thought to enter mainly via the portal circulation and disseminate throughout the body. The most common extra-intestinal site is the liver, occasionally presenting as a medical emergency.^{72, 75} Invasion of liver tissue may occur after symptomatic intestinal amoebiasis or in cases where the colonic infection is asymptomatic. Nearly half of all patients with amoebic liver abscesses do not have a history suggestive of amoebic colitis.

Hepatic amoebiasis is a slowly progressive, insidious disease that typically begins as a nonspecific febrile illness, with pain and tenderness in the right upper quadrant of the abdomen. Frequently, this presents as referred pain in the shoulder region. Examination at that time may reveal only a slightly enlarged, tender liver or a mass. Sometimes, the so-called ‘intercostal sign,’ pressure exerted between the ribs, will detect tenderness.⁹ Most patients with hepatic amoebiasis have involvement of the right hepatic lobe, but the left lobe of the liver can also be



Figure 12.6. Portion of transverse colon showing extensive ulceration due to intestinal infection with *E. histolytica*.

infected; the enlargement and tenderness can be central or even left-sided.

The lungs are the following most common extra-intestinal sites of infection.^{76, 77} Direct extension to the right pleural space and the lung is the most common form of intrathoracic amoebiasis. Hematogenous spread may cause metastatic amoebiasis in other portions of the lung and the pleura, as well as in other organs, notably the brain. Amoebic pericarditis can occur in the same manner. The major pleuropulmonary manifestations include effusion, pleurisy, empyema, and lung abscess. Occasionally, a hepatobronchial fistula forms, resulting in a productive cough with large amounts of amoebae-containing necrotic material. Embolism is rare. Rupture into the pericardium is usually fatal.

Cerebral amoebiasis rarely occurs. The onset is usually abrupt and is associated with a high mortality rate unless diagnosed early in the infection.⁷⁸

It is now recognized that many populations infected with HIV-1 have higher exposures and asymptomatic colonization rates for *E. histolytica*. It is unclear whether there is a significantly higher rate of progression to invasive disease in these patients compared to those without HIV infection. Still, the higher incidence in this population increases the number of infected individuals potentially progressing to invasive disease.⁷⁹⁻⁸²

Diagnosis (see Clinical Appendix)

The site, as well as the duration of infection, dictates the most appropriate diagnostic test. Stool testing for heme is positive in almost all cases of intestinal amoebiasis if performed rigorously. In contrast, stool tests for leukocytes tend to be negative due to the ability of the amoeba to destroy leukocytes

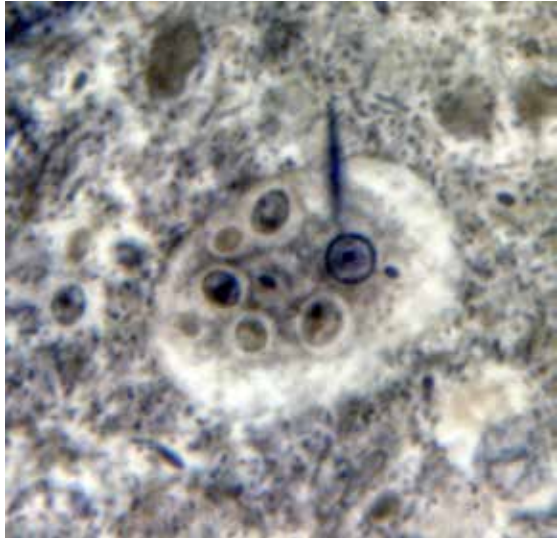


Figure 12.7. Trophozoite of *E. histolytica* in stool of patient suffering from amoebic dysentery. Note Charcot-Leyden crystal “pointing” to nucleus. Also note numerous red cells in parasite cytoplasm. 30 μ m.

despite the invasive nature of this disease. Definitive diagnosis depends upon detecting antigens in stool, NAAT on stool or tissue samples, or microscopy coupled with species identification by one of the first two testing modalities.^{12, 83} Antigen detection and NAATs are replacing microscopy based on their sensitivity, specificity, rapidity, ease of execution, and cost. An ELISA-based test is now in common use that is both rapid and specific for distinguishing *E. histolytica* from its usually non-pathogenic *doppelgänger*s.¹² Testing with NAAT is reported to have a sensitivity



Figure 12.8. Charcot-Leyden crystal in stool of patient suffering from amoebic dysentery. These crystals can also be found in patients infected with *Trichuris trichiura* and *Strongyloides stercoralis*.

of 100 percent, greater than stool antigen testing. It is now available in many centers as part of a gastrointestinal (GI) diagnostic panel.^{79, 84}

Microscopy is still the only diagnostic modality in many facilities. Stool culture is not clinically available and does not play a role in the routine diagnosis of this disease. The trophozoites of *E. histolytica* are more often described as showing evidence of erythrophagocytosis, and this has been suggested to allow for definitive diagnosis using microscopy. There are both *in vitro* and *in vivo* reports that nonpathogenic amoebae phagocytize red cells, and in one study, over 15% of cases of *E. dispar* showed erythrophagocytosis on stool microscopy.⁸⁵⁻⁸⁸ If red blood cells are seen in the cytoplasm, particularly if there are more than two per trophozoite, then *E. histolytica* is more likely (Fig. 12.7).⁸⁸

The Charcot-Leyden crystals (Fig. 12.8) in stool are frequently present when patients suffer from disease caused by *E. histolytica*, but they are also seen with heavy infection caused by *Trichuris trichiura* and *Strongyloides stercoralis* and are, therefore, not pathognomonic for amoebiasis. PCR can also help diagnose liver disease when used on aspirates derived from the abscess.⁸⁹

Since infection with *E. histolytica* invariably leads to long-lasting antibody production, antibody-based tests are sometimes difficult to interpret, especially during chronic infection.⁹⁰ Serological testing will become positive, even in intestinal amoebiasis infection, 5–7 days after onset of symptoms. It usually remains positive for years, consequently limiting their utility in diagnosing infections in endemic areas where up to 35% of the population may have seropositivity due to prior exposure.⁹¹ The indirect hemagglutination assay (IHA) and indirect fluorescent antibody (IFA) test are used together to rule out

the possibility of extra-intestinal disease but are not definitive proof of infection. Intestinal amoebiasis must always be considered in patients with protracted diarrhea and dysentery. The diagnosis must also be considered in patients presenting with intraluminal colonic masses because of the development of amoebomas resembling colon carcinoma. In extra-intestinal amoebiasis, identification of the lesion by the various modalities and the presence of a travel history compatible with amoebiasis, in parallel with the identification of amoebae in the colon, points to the diagnosis.

Extra-intestinal amoebiasis is often a more challenging diagnosis. Intestinal and extra-intestinal amoebiasis do not usually occur simultaneously, and stool testing is usually negative in extra-intestinal amoebiasis. Serology is usually positive in cases of extra-intestinal amoebiasis but may be negative during the first seven days. Imaging tests play a critical role in the diagnosis of extra-intestinal amoebiasis. Radiography of the abdomen may show enlargement of the liver and a fixed, raised diaphragm. In cases of perforation of the diaphragm, there may be evidence of consolidation of one of the lower lobes of the lung or its lower segment and a pleural effusion. A radionuclide or a CT scan often reveals the abscess; they may also show additional abscesses; however, these are rare. An amoebic liver abscess on ultrasonography usually appears as a round hypodense area contiguous to the liver capsule, usually without significant wall echoes.⁹² Direct extension to the right pleural space and the lung is the most common form of intrathoracic amoebiasis, but hematogenous spread may cause metastatic amoebiasis in other portions of the lung and the pleura, as well as in other organs, notably the brain. Amoebic pericarditis can occur in the same manner.⁹³

The cyst can be aspirated if the diagnosis

remains doubtful after serological testing and imaging. The obtained fluid from a hepatic amoebic abscess is sometimes a brown fluid containing necrotic hepatocytes that has been likened to anchovy paste. Still, in many cases, the fluid may be clear, yellow, or described as purulent.⁹⁴ Amoebic forms are not always present, and antigen testing or NAAT helps confirm the diagnosis.⁹⁵ Abscesses may be secondarily infected with bacteria, so sending the fluid for bacterial culture is recommended.^{96, 97} Although eosinophils may play some role in local tissue control early in the course of hepatic amoebiasis, very few patients have elevated eosinophils in the peripheral circulation, and patients may even become eosinopenic in severe infections.⁹⁸⁻¹⁰⁰

Treatment (see Clinical Appendix)

All forms of symptomatic amoebiasis are considered invasive and, consequently, should be treated with an agent that is active against the tissue invasive forms and reaches adequate levels at the sites of infection. Metronidazole is the drug of choice for intestinal and extra-intestinal infections.¹⁰¹ It can be given in equivalent doses orally or intravenously due to its high oral bioavailability. This drug also has a few limitations and some adverse side effects. Use of alcohol is prohibited during treatment, as it induces a side effect similar to that caused by disulfiram (Antabuse) therapy. Alternative agents include tinidazole and second-line agents that may be inferior in efficacy, such as nitazoxanide or ornidazole. While ten days of metronidazole may eliminate the intraluminal infection, clearing the cyst stage is not always successful. A second intraluminal agent to target the intraluminal cysts has been recommended. Treatment of asymptomatic carriers of *E. histolytica* to prevent disease or transmission is controversial.¹⁰² A cysticidal intraluminal agent alone may be adequate for asymptomatic cyst passers and those with non-dysenteric amoebic colitis.

The intraluminal agent paromomycin can be used for this indication, while other agents, such as diloxanide furoate and iodoquinol (diiodohydroxyquinoline), are effective at killing cysts and can be used.¹⁰³ Additional antibiotics are appropriate in patients with suspected secondary bacterial infection.^{96, 97}

While metronidazole remains the recommended therapy, metronidazole-resistant strains of *E. histolytica* can be induced under laboratory conditions.^{104, 105} Liver abscesses resolve slowly despite treatment with the recommended high doses of metronidazole. Aspiration of an amoebic liver abscess may serve to confirm the diagnosis and as a therapeutic intervention. There are established indications for therapeutic drainage of an amoebic liver abscess and surgery in severe colitis cases. A surgeon or interventional radiologist should be involved: 1. if there is no clinical improvement within 48–72 hours despite appropriate medical therapy, 2. for abscesses greater than 10 cm in diameter, 3. when there is marked elevation of the diaphragm, 4. for abscesses in the left lobe, and 5. when there is negative serology, which might raise suspicion of a pyogenic abscess.^{72,}

^{106, 107}

There are few reports of patients surviving amoebic abscess of the brain.⁷⁸ In the case of infection involving the pericardium, quick aspiration of an expanding pericardial effusion, combined with aggressive anti-amoebic therapy, has saved the lives of most of those suffering from this rare manifestation of the infection.¹⁰⁸

Prevention and Control

Good public health practices, starting with ensuring the safety of drinking water supplies and, in some cases, watershed management, are the best long-term approaches to controlling most waterborne

diarrheal disease agents. Screening of food handlers with periodic stool examinations can identify carriers. Recurrent outbreaks of amoebiasis in mental institutions can be prevented by strictly adhering to appropriate sanitary practices coupled with routine stool examinations of the patients. Treating all infected individuals likely reduces onward transmission.

Vaccine targets against both intestinal and extra-intestinal infection have been identified. Ongoing research on vaccines targeting membrane and non-membrane targets continues and may one day be available for the highest-risk groups, including children in endemic areas and other high-risk groups such as travelers.^{109, 110}

References

1. Bercu, T. E., et al., Amebic colitis: new insights into pathogenesis and treatment. *Curr Gastroenterol Rep* **2007**, 9 (5), 429-33.
2. Heredia, R. D., et al., *Entamoeba moshkovskii* perspectives of a new agent to be considered in the diagnosis of amebiasis. *Acta Trop* **2012**, 123 (3), 139-45.
3. da Silva, C. A. V., et al., South American *Entamoeba dispar* strains produce amoebic liver abscesses with different pathogenicities and evolutionary kinetics. *Acta Trop* **2021**, 224, 106114.
4. Ali, I. K., et al., *Entamoeba moshkovskii* infections in children, Bangladesh. *Emerg Infect Dis* **2003**, 9 (5), 580-4.
5. Royer, T. L., et al., *Entamoeba bangladeshi* nov. sp., Bangladesh. *Emerg Infect Dis* **2012**, 18 (9), 1543-5.
6. Singh, A., et al., Epidemiology of clinically relevant *Entamoeba* spp. (*E. histolytica*/*dispar*/*moshkovskii*/*bangladeshi*): A cross sectional study from North India. *PLoS Negl Trop Dis* **2021**, 15 (9), e0009762.
7. Marciano-Cabral, F., Free-living amoebae as agents of human infection. *J Infect Dis* **2009**, 199 (8), 1104-6.
8. Kotloff, K. L., et al., Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* **2013**, 382 (9888), 209-22.
9. Haque, R., et al., Amebiasis. *N Engl J Med* **2003**, 348 (16), 1565-73.
10. Ngui, R., et al., Copro-molecular study of *Entamoeba* infection among the indigenous community in Malaysia: A first report on the species-specific prevalence of *Entamoeba* in dogs. *Acta Trop* **2020**, 204, 105334.
11. Tannich, E., Royal Society of Tropical Medicine and Hygiene Meeting at Manson House, London, 19 February 1998. Amoebic disease. *Entamoeba histolytica* and *E. dispar*: comparison of molecules considered important for host tissue destruction. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1998**, 92 (6), 593-6.
12. Anane, S.; Khaled, S., [*Entamoeba histolytica* and *Entamoeba dispar*: differentiation methods and implications]. *Annales de biologie clinique* **2005**, 63 (1), 7-13.
13. Blessmann, J., et al., Real-time PCR for detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in fecal samples. *J Clin Microbiol* **2002**, 40 (12), 4413-7.
14. Reed, S., et al., Amoebiasis In: *Infections of the Gastrointestinal Tract* 2nd ed. 2002; p 961-978.
15. Martinez-Paloma, A., et al., Topley and Wilson's Microbiology and Microbial Infections 9th ed. 1998; p 157-177.
16. Loftus, B., et al., The genome of the protist parasite *Entamoeba histolytica*. *Nature* **2005**, 433 (7028), 865-8.
17. Losch, F. A., Massenhafte Entwicklung von Amoben in Dickdarm. *Arch Pathol Anat Phys KIm Med Virchow* **1875**, 65, 196-211.
18. Tanyuksel, M.; Petri, W. A., Jr., Laboratory diagnosis of amebiasis. *Clin Microbiol Rev* **2003**, 16 (4), 713-29.
19. Cox, F. E., History of human parasitology. *Clin Microbiol Rev* **2002**, 15 (4), 595-612.
20. Quincke, H. I.; Roos, E., Uber Amoben-enteritis. *Berl KIm Wochenschr* **1893**, 30 1089-1094.

21. Schaudinn, F., Untersuchungen über Fortpflanzung einiger Rhizopoden Berlin, Verlag Von Julius Springer in Berlin: 1903.
22. Councilman, W. T.; Lafleur, H. A., Amoebic dysentery. *Johns Hopkins Hosp Rep* **1891**, 2, 395-548.
23. Boeck, W. C.; J., Cultivation of *Entamoeba histolytica*. *Am* **1925**, 5, 371-407.
24. Dobell, C., Researches on the intestinal protozoa of monkeys and man. *Parasitology* **1928**, 20, 357-412.
25. Saavedra, E., et al., Glycolysis in *Entamoeba histolytica*. Biochemical characterization of recombinant glycolytic enzymes and flux control analysis. *The FEBS journal* **2005**, 272 (7), 1767-83.
26. Mai, Z., et al., Hsp60 is targeted to a cryptic mitochondrion-derived organelle ("crypton") in the microaerophilic protozoan parasite *Entamoeba histolytica*. *Molecular and cellular biology* **1999**, 19 (3), 2198-205.
27. Tovar, J., et al., The mitosome, a novel organelle related to mitochondria in the amitochondrial parasite *Entamoeba histolytica*. *Molecular microbiology* **1999**, 32 (5), 1013-21.
28. Nakada-Tsukui, K.; Nozaki, T., Molecular Basis of the Trafficking of Cysteine Proteases and Other Soluble Lysosomal Proteins in *Entamoeba histolytica*. *Amebiasis Springer Japan* **2015**.
29. Aguilera, P., et al., *Entamoeba histolytica* mitosomes: organelles in search of a function. *Exp Parasitol* **2008**, 118 (1), 10-6.
30. Bakatselou, C., et al., Analysis of genes of mitochondrial origin in the genus *Entamoeba*. *The Journal of eukaryotic microbiology* **2003**, 50 (3), 210-4.
31. Gozalez, E., et al., Calreticulin-like molecule in trophozoites of *Entamoeba histolytica*. *Am Med Hyg* **2002**, 67, 636-639.
32. Teixeira, J. E.; Huston, C. D., Evidence of a continuous endoplasmic reticulum in the protozoan parasite *Entamoeba histolytica*. *Eukaryot Cell* **2008**, 7 (7), 1222-6.
33. Walker, E. L., et al., Experimental entamoebic dysentery. *Phillipine Med* **1913**, 8, 253-330.
34. Makioka, A., et al., Expression analysis of *Entamoeba invadens* profilins in encystation and excystation. *Parasitol Res* **2012**, 110 (6), 2095-104.
35. Eichinger, D., A role for a galactose lectin and its ligands during encystment of *Entamoeba*. *J Eukaryot Microbiol* **2001**, 48 (1), 17-21.
36. Ravdin, J. I.; Guerrant, R. L., Role of adherence in cytopathogenic mechanisms of *Entamoeba histolytica*. Study with mammalian tissue culture cells and human erythrocytes. *J Clin Invest* **1981**, 68 (5), 1305-13.
37. Saffer, L. D.; Petri, W. A., Jr., Role of the galactose lectin of *Entamoeba histolytica* in adherence-dependent killing of mammalian cells. *Infect Immun* **1991**, 59 (12), 4681-3.
38. Makioka, A., et al., Effect of proteasome inhibitors on the growth, encystation, and excystation of *Entamoeba histolytica* and *Entamoeba invadens*. *Parasitology research* **2002**, 88 (5), 454-9.
39. Eichinger, D., Encystation of entamoeba parasites. *BioEssays : news and reviews in molecular, cellular and developmental biology* **1997**, 19 (7), 633-9.
40. Pires-Santos, G. M., et al., Optimization of *Entamoeba histolytica* culturing in vitro. *Exp Parasitol* **2012**, 132 (4), 561-5.
41. Carrero, J. C.; Lacleste, J. P., Molecular biology of *Entamoeba histolytica*: a review. *Archives of medical research* **1996**, 27 (3), 403-12.
42. Lohia, A., The cell cycle of *Entamoeba histolytica*. *Molecular and cellular biochemistry* **2003**, 253 (1-2), 217-22.
43. Petrin, D., et al., Clinical and microbiological aspects of *Trichomonas vaginalis*. *Clinical microbiology reviews* **1998**, 11 (2), 300-17.
44. Stauffer, W.; Ravdin, J. I., *Entamoeba histolytica*: an update. *Current opinion in infectious diseases* **2003**, 16 (5), 479-85.
45. Begum, S., et al., Immune Evasion Mechanisms of *Entamoeba histolytica*: Progression to Disease. *Front Microbiol* **2015**, 6, 1394.
46. Dodson, J. M., et al., Infection and immunity mediated by the carbohydrate recognition domain of the *Entamoeba histolytica* Gal/GalNAc lectin. *The Journal of infectious diseases* **1999**, 179 (2), 460-6.

47. Rosales-Encina, J. L., et al., Isolation of a 220-kilodalton protein with lectin properties from a virulent strain of *Entamoeba histolytica*. *The Journal of infectious diseases* **1987**, 156 (5), 790-7.
48. Vines, R. R., et al., Regulation of adherence and virulence by the *Entamoeba histolytica* lectin cytoplasmic domain, which contains a beta2 integrin motif. *Molecular biology of the cell* **1998**, 9 (8), 2069-79.
49. Braga, L. L., et al., Inhibition of the complement membrane attack complex by the galactose-specific adhesion of *Entamoeba histolytica*. *The Journal of clinical investigation* **1992**, 90 (3), 1131-7.
50. Kain, K. C.; Ravdin, J. I., Galactose-specific adhesion mechanisms of *Entamoeba histolytica*: model for study of enteric pathogens. *Methods in enzymology* **1995**, 253, 424-39.
51. Arias-Negrete, S., et al., Expression of *in vitro* virulence by *Entamoeba histolytica*: effect of calmodulin inhibitors. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica* **1999**, 107 (9), 875-81.
52. McKerrow, J. H., et al., The proteases and pathogenicity of parasitic protozoa. *Annual review of microbiology* **1993**, 47, 821-53.
53. Ralston, K. S., et al., Trophocytosis by *Entamoeba histolytica* contributes to cell killing and tissue invasion. *Nature* **2014**, 508 (7497), 526-30.
54. Brandt, H.; Tamayo, R. P., Pathology of human amebiasis. *Hum Pathol* **1970**, 1 (3), 351-85.
55. Reed, S. L., et al., The extracellular neutral cysteine proteinase of *Entamoeba histolytica* degrades anaphylatoxins C3a and C5a. *Journal of immunology (Baltimore, Md. : 1950)* **1995**, 155 (1), 266-74.
56. Franco, E., et al., Specific and reversible inhibition of *Entamoeba histolytica* cysteine-proteinase activities by Zn²⁺: implications for adhesion and cell damage. *Archives of medical research* **1999**, 30 (2), 82-8.
57. Leippe, M.; Muller-Eberhard, H. J., The pore-forming peptide of *Entamoeba histolytica*, the protozoan parasite causing human amoebiasis. *Toxicology* **1994**, 18, 1-3.
58. Vaccaro, A. M., et al., Saposins and their interaction with lipids. *Neurochemical research* **1999**, 24 (2), 307-14.
59. Ravdin, J. I., et al., Relationship of free intracellular calcium to the cytolytic activity of *Entamoeba histolytica*. *Infection and immunity* **1988**, 56 (6), 1505-12.
60. Yu, Y.; Chadee, K., Secreted *Entamoeba histolytica* proteins stimulate interleukin-8 mRNA expression and protein production in human colonic epithelial cells. *Archives of medical research* **1997**, 28 Spec No, 223-4.
61. Utrera-Barillas, D., et al., An anti-inflammatory oligopeptide produced by *Entamoeba histolytica* down-regulates the expression of pro-inflammatory chemokines. *Parasite immunology* **2003**, 25 (10), 475-82.
62. Choudhuri, G., et al., Protective immunity to *Entamoeba histolytica* infection in subjects with antiamebic antibodies residing in a hyperendemic zone. *Scandinavian journal of infectious diseases* **1991**, 23 (6), 771-6.
63. Lotter, H., et al., Identification of an epitope on the *Entamoeba histolytica* 170-kD lectin conferring antibody-mediated protection against invasive amebiasis. *The Journal of experimental medicine* **1997**, 185 (10), 1793-801.
64. Ravdin, J. I.; Kelsall, B. L., Role of mucosal secretory immunity in the development of an amebiasis vaccine. *The American journal of tropical medicine and hygiene* **1994**, 50 (5 Suppl), 36-41.
65. Abou-el-Magd, I., et al., Humoral and mucosal IgA antibody response to a recombinant 52-kDa cysteine-rich portion of the *Entamoeba histolytica* galactose-inhibitable lectin correlates with detection of native 170-kDa lectin antigen in serum of patients with amebic colitis. *The Journal of infectious diseases* **1996**, 174 (1), 157-62.
66. Stanley, S. L. J., Protective immunity to amoebiasis: new insights and new challenges. *Dis* **2001**, 184, 504-6.
67. Ghadirian, E.; Denis, M., *In vivo* activation of macrophages by IFN-gamma to kill *Entamoeba histolytica* trophozoites in vitro. *Parasite immunology* **1992**, 14 (4), 397-404.

68. Seguin, R., et al., The tumor necrosis factor alpha-stimulating region of galactose-inhibitable lectin of *Entamoeba histolytica* activates gamma interferon-primed macrophages for amebicidal activity mediated by nitric oxide. *Infect Immun* **1997**, *65* (7), 2522-7.
69. Nagata, N., et al., Risk factors for intestinal invasive amebiasis in Japan, 2003-2009. *Emerg Infect Dis* **2012**, *18* (5), 717-24.
70. Ravdin, J. I., et al., Antigenicity, immunogenicity and vaccine efficacy of the galactose-specific adherence protein of *Entamoeba histolytica*. *Vaccine* **1993**, *11* (2), 241-6.
71. Marinets, A., et al., Protection against invasive amebiasis by a single monoclonal antibody directed against a lipophosphoglycan antigen localized on the surface of *Entamoeba histolytica*. *The Journal of experimental medicine* **1997**, *186* (9), 1557-65.
72. Wells, C. D.; Arguedas, M., Amebic liver abscess. *Southern medical journal* **2004**, *97* (7), 673-82.
73. Das, S. K., et al., Comparison of clinical and laboratory characteristics of intestinal amebiasis with shigellosis among patients visiting a large urban diarrheal disease hospital in Bangladesh. *Am J Trop Med Hyg* **2013**, *89* (2), 339-44.
74. Parshad, S., et al., Primary cutaneous amoebiasis: case report with review of the literature. *International journal of dermatology* **2002**, *41* (10), 676-80.
75. Hoffner, R. J., et al., Common presentations of amebic liver abscess. *Annals of emergency medicine* **1999**, *34* (3), 351-5.
76. Lyche, K. D.; Jensen, W. A., Pleuropulmonary amebiasis. *Seminars in respiratory infections* **1997**, *12* (2), 106-12.
77. Mbaye, P. S., et al., [Pleuropulmonary manifestations of amebiasis]. *Revue de pneumologie clinique* **1998**, *54* (6), 346-52.
78. Sundaram, C., et al., Brain abscess due to *Entamoeba histolytica*. *The Journal of the Association of Physicians of India* **2004**, *52*, 251-2.
79. Hung, C.-C., et al., Invasive amebiasis as an emerging parasitic disease in patients with human immunodeficiency virus type 1 infection in Taiwan. *Archives of internal medicine* **2005**, *165* (4), 409-15.
80. Hung, C. C., et al., Increased risk for *Entamoeba histolytica* infection and invasive amebiasis in HIV seropositive men who have sex with men in Taiwan. *PLoS Negl Trop Dis* **2008**, *2* (2), e175.
81. Park, W. B., et al., Amebic liver abscess in HIV-infected patients, Republic of Korea. *Emerg Infect Dis* **2007**, *13* (3), 516-7.
82. Wu, K. S., et al., Comparison of clinical characteristics of amebic liver abscess in human immunodeficiency virus (HIV)-infected and non-HIV-infected patients. *J Microbiol Immunol Infect* **2008**, *41* (6), 456-61.
83. Mirelman, D., et al., Comparison of use of enzyme-linked immunosorbent assay-based kits and PCR amplification of rRNA genes for simultaneous detection of *Entamoeba histolytica* and *E. dispar*. *Journal of clinical microbiology* **1997**, *35* (9), 2405-7.
84. Buss, S. N., et al., Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. *J Clin Microbiol* **2015**, *53* (3), 915-25.
85. Talamas-Lara, D., et al., Erythrophagocytosis in *Entamoeba histolytica* and *Entamoeba dispar*: a comparative study. *Biomed Res Int* **2014**, *2014*, 626259.
86. Boettner, D. R., et al., *Entamoeba histolytica* and *Entamoeba dispar* utilize externalized phosphatidylserine for recognition and phagocytosis of erythrocytes. *Infect Immun* **2005**, *73* (6), 3422-30.
87. Gonzalez-Ruiz, A., et al., Value of microscopy in the diagnosis of dysentery associated with invasive *Entamoeba histolytica*. *J Clin Pathol* **1994**, *47* (3), 236-9.
88. Haque, R., et al., Rapid diagnosis of *Entamoeba* infection by using *Entamoeba* and *Entamoeba histolytica* stool antigen detection kits. *J Clin Microbiol* **1995**, *33* (10), 2558-61.
89. Zengzhu, G., et al., Analysis by enzyme-linked immunosorbent assay and PCR of human liver abscess aspirates from patients in China for *Entamoeba histolytica*. *Journal of clinical microbiology* **1999**, *37* (9), 3034-6.

90. Lotter, H., et al., Evaluation of three serological tests for the detection of antiamebic antibodies applied to sera of patients from an area endemic for amebiasis. *Tropical medicine and parasitology : official organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ)* **1995**, 46 (3), 180-2.
91. Samie, A., et al., Prevalence and species distribution of *E. Histolytica* and *E. Dispar* in the Venda region, Limpopo, South Africa. *Am J Trop Med Hyg* **2006**, 75 (3), 565-71.
92. Petri, W. A., Jr., Pathogenesis of amebiasis. *Curr Opin Microbiol* **2002**, 5 (4), 443-7.
93. Shamsuzzaman, S. M.; Hashiguchi, Y., Thoracic amebiasis. *Clinics in chest medicine* **2002**, 23 (2), 479-92.
94. Gaut, D., et al., Hepatic Abscess in a Returning Traveler with Crohn's Disease: Differentiating Amebic from Pyogenic Liver Abscess. *Case Rep Med* **2018**, 2018, 9593865.
95. Weitzel, T., et al., Enteric multiplex PCR panels: A new diagnostic tool for amoebic liver abscess? *New Microbes New Infect* **2017**, 18, 50-53.
96. Cosme, A., et al., Pyogenic versus amoebic liver abscesses. A comparative clinical study in a series of 58 patients. *Rev Esp Enferm Dig* **2010**, 102 (2), 90-9.
97. Dhamrah, U., et al., Bacterial Superinfection of Amoebic Liver Abscess. *J Investig Med High Impact Case Rep* **2020**, 8, 2324709620926900.
98. Lopez-Osuna, M., et al., [The eosinophil and *Entamoeba histolytica*. II. Testicular amebic lesions produced in eosinophilic rats]. *Arch Invest Med (Mex)* **1990**, 21 Suppl 1, 263-5.
99. Lopez-Osuna, M., et al., Does the eosinophil have a protective role in amebiasis? *Mem Inst Oswaldo Cruz* **1997**, 92 Suppl 2, 237-40.
100. Schulte, C., et al., Diagnostic significance of blood eosinophilia in returning travelers. *Clin Infect Dis* **2002**, 34 (3), 407-11.
101. Rosenblatt, J. E., Antiparasitic agents. *Mayo Clinic proceedings* **1992**, 67 (3), 276-87.
102. Blessmann, J., et al., Longitudinal study of intestinal *Entamoeba histolytica* infections in asymptomatic adult carriers. *J Clin Microbiol* **2003**, 41 (10), 4745-50.
103. McAuley, J. B.; Herwaldt, B. L., Diloxanide furoate for treating asymptomatic *Entamoeba histolytica* cyst passers: 14 years' experience in the United States. *Clin Infect Dis* **1992**, 15, 464-468.
104. Bansal, D., et al., *In vitro* activity of antiamoebic drugs against clinical isolates of *Entamoeba histolytica* and *Entamoeba dispar*. *Annals of clinical microbiology and antimicrobials* **2004**, 3, 27.
105. Upcroft, P.; Upcroft, J. A., Drug targets and mechanisms of resistance in the anaerobic protozoa. *Clinical microbiology reviews* **2001**, 14 (1), 150-64.
106. Gibney, E. J., Amoebic liver abscess. *The British journal of surgery* **1990**, 77 (8), 843-4.
107. de la Rey Nel, J., et al., Indications for aspiration of amoebic liver abscess. *S Afr Med J* **1989**, 75 (8), 373-6.
108. Kirchhoff, L. V., et al., Parasitic diseases of the heart. *Frontiers in bioscience* **2004**, 9, 706-23.
109. Quach, J., et al., The future for vaccine development against *Entamoeba histolytica*. *Hum Vaccin Immunother* **2014**, 10 (6), 1514-21.
110. Jasni, N., et al., *Entamoeba histolytica*: Membrane and Non-Membrane Protein Structure, Function, Immune Response Interaction, and Vaccine Development. *Membranes (Basel)* **2022**, 12 (11).

13. *Balantidium coli* / *Balantidioides coli*

(Malmsten 1857)

Pronunciation: \bal-ən-'tid-ē-əm\kō-,lī\ /
 \bal-ən-'tid-ē-oy- dēs \kō-,lī\

Introduction

Balantidium coli (\BAL-an-TID-ee-um\ KOHL-eye\)) is the only ciliated protozoan that routinely infects humans. There is a suggestion that there is a need for taxonomic revision, changing the name of this organism to *Balantidioides coli* (\BAL-an-TID-ee-oy-dees\KOHl-eye\)) or *Neobalantidium coli* (\Nee-ō-BAL-an-TID-ee-um\KOHl-eye\). Still, the medical community does not widely accept this change.^{1,2} Balantidiasis, or neobalantidiasis, occurs worldwide, but the prevalence of human infection is unknown. It is endemic in Japan, New Guinea, Micronesia, Seychelles Islands, Thailand, South Africa, Central and South America, and Europe.³⁻⁸ Molecular studies suggest the previously named *Balantidium suis* in pigs, *Balantidium caviae* in guinea pigs, *Balantidium wenrichi*, *Balantidium philippinensis*, and *Balantidium cunhamunizi* in non-human primates, and *Balantidium struthionis* in ostriches are the same organism, *Balantidium coli*.^{9, 10, 11}



Figure 13.1. Trophozoite of *Balantidium coli*. Note large macronucleus and cytostome (arrow). 150 μ m.

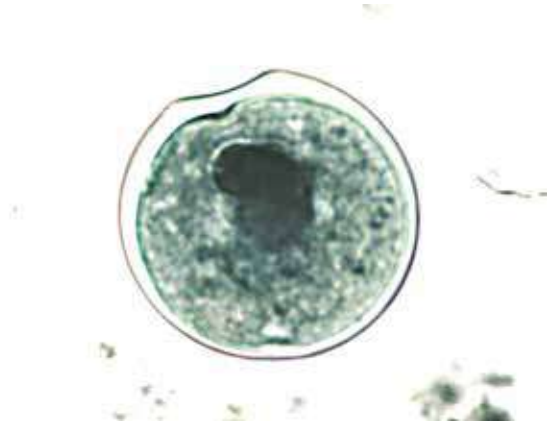


Figure 13.2. Cyst of *Balantidium coli*. Note macronucleus. 65 μ m.

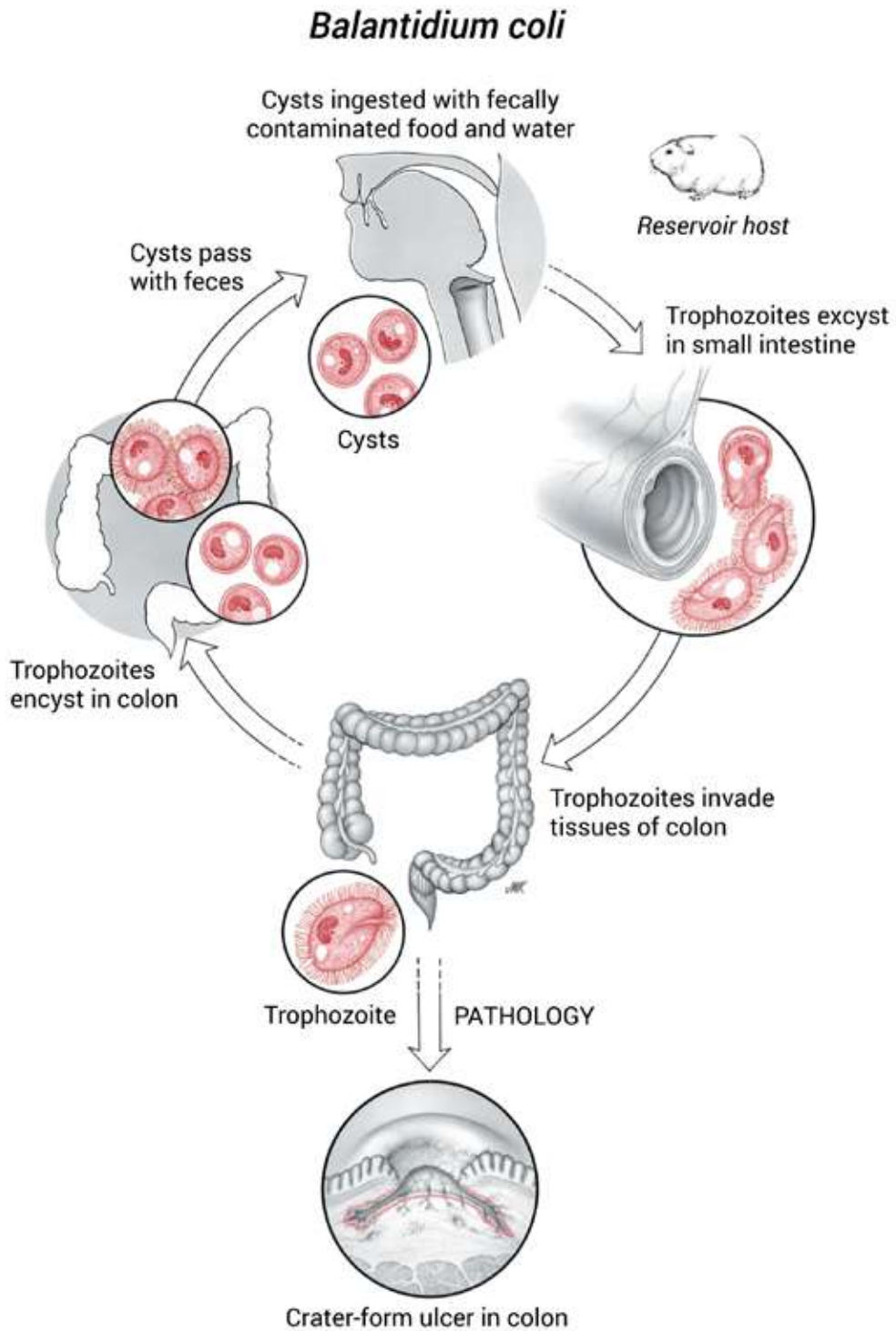
Sporadic epidemics have occurred in institutionalized populations. *B. coli* locates to the large intestine, where it causes dysentery, occasionally leading to fatalities. It has many reservoir hosts, including domestic and wild mammals (non-human primates, guinea pigs, horses, cattle, pigs, wild boars, and rats).³ When patients suffering from HIV encounter *B. coli*, the infection can spread beyond the gastrointestinal (GI) tract.¹²

Historical Information

In 1857, Pehr Malmsten described two patients from Stockholm, Sweden, suffering from acute diarrheal disease with *B. coli* organisms in their feces.¹³ One patient recovered, while the other succumbed to the infection. Four years later, in 1861, Rudolph Leuckart described this same organism that he isolated from the intestine of a pig.¹¹ F. Stein equated these two isolates, and they were both named *B. coli*.^{11, 14}

Life Cycle

B. coli produces two stages: the trophozoite (Fig. 13.1) and the cyst (Fig. 13.2). The invasive stage is the trophozoite. *B. coli* resides in the tissues of the large intestine (Fig. 13.3) in a similar habitat to that of *Entamoeba histo-*



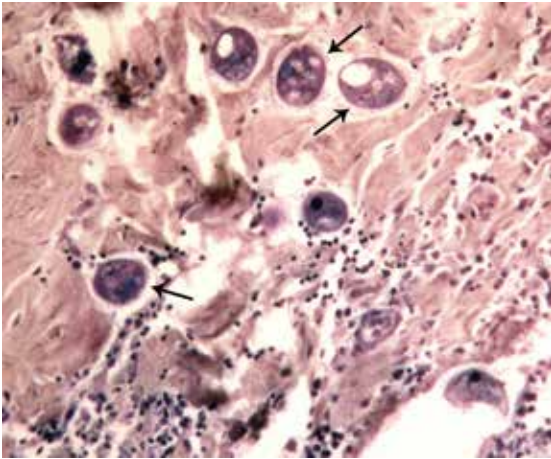


Figure 13.3. Histologic section of large intestine infected with *B. coli* (arrows).

lytica (Figs. 12.4, 12.6), from which it must be clinically distinguished. The trophozoite of *B. coli* ingests living cells and causes ulcerations to develop at the site of infection. The cyst of *Entamoeba histolytica* is only 10–20 μm in diameter and is often present in loose stools. In contrast, the cyst stage of *B. coli* measures 65 μm in diameter and is usually only seen in formed stools.¹¹

Infection begins by ingesting the cyst in fecally contaminated food or beverages. The trophozoite excysts in the small intestine then relocates to the large intestine. The preferred site of infection is the epithelium of the transverse and descending colon. *B. coli* is usually limited to the bowel, although a case involving a liver abscess has been reported, and infection in the lungs and heart has been reported.^{8, 15, 16} The trophozoite divides by simple binary fission within the host, but in culture, it behaves like all other free-living ciliates undergoing syngamy, a specialized sexual reproduction similar to conjugation.¹⁷

The trophozoites cause extensive destruction to the surrounding tissue (Fig. 13.3). During prolonged infection, some trophozoites enter the lumen of the colon, where they secrete an impervious, hyaline, acellular layer resulting

in the formation of the cyst stage. The cyst exits from the host in the fecal mass and is immediately infectious without requiring an intermediate host, allowing for direct human-to-human transmission. In many ecological settings, pigs are the reservoirs since infection is more common where pigs live in close association with human habitats, and several outbreaks have been associated with human exposure to pig feces, such as the outbreak in Truk.¹⁸ Guinea pigs are thought to harbor *B. coli* as a commensal and have been the source of some human infections.⁸

Cellular and Molecular Pathogenesis

B. coli survives in anaerobic and aerobic conditions and uses carbohydrates as its primary energy source.¹¹ The trophozoite possesses a hyaluronidase, which is presumed to dissolve extracellular matrix components and disrupt the mucosal epithelial cells.¹⁹ Proteases, most likely lysosomal in origin, released within food vacuoles (phagolysosomes), participate in the digestion of cell debris that enters through the peristome (mouth-like opening) located at the narrowed end of the trophozoite.

Clinical Disease

The four main presentations that can result after human exposure to *B. coli* are a lack of symptoms with carriage or clearance, acute colitis, chronic infection, and invasive disease.^{6, 16, 20–22} Although most exposed individuals appear asymptomatic, a minority will develop acute balantidiasis with watery diarrhea or dysentery.¹⁶ Fever, nausea, vomiting, and asthenia (physical weakness or lack of energy) have been described, and some deaths have been associated with intestinal perforation and consequent sepsis.²¹ Chronic infection has been studied in endemic areas

and may be responsible for negative impacts on growth in infected children.²³ Rarely, *B. coli* causes ulcerative and granulomatous disease in the colon and appendix, leading to typhlitis (inflammation of the cecum) and appendicitis.²⁴ Individuals who are immune compromised due to malnutrition, HIV infection, or other causes may develop invasive disease with organisms invading the lungs, urinary tract, liver, and heart.^{8, 12, 15, 16, 25}

Diagnosis (see Clinical Appendix)

Definitive diagnosis is by identifying the organism (trophozoite or cyst; Figs 13.1, 13.2) by microscopy in a stool sample or on a stained section of tissue from a biopsy of an ulcer identified by colonoscopy.²⁶ One might find trophozoites in freshly obtained watery stools, while only the cyst stage is present in formed stools. There are no established diagnostic serological or molecular tests available.²⁷ Culture is also not routinely used for diagnosis, so microscopy on stool specimens is the only diagnostic test.²⁷

Treatment (see Clinical Appendix)

Although there are a limited number of studies to guide therapy, tetracycline, metronidazole, iodoquinol, paromomycin, nitazoxanide, and chloroquine have been used to treat balantidiasis.^{11, 26, 28} Surgery (bowel resection) is sometimes necessary in severe cases of dysentery.

Prevention and Control

Good sanitation and a clean source of drinking water are prerequisites for controlling the spread of *B. coli*. The usual chlorine concentration used to purify water is inadequate to destroy the cyst stage of *B. coli*.¹¹ Domestic pigs would need to be restricted from releasing infective cysts into waters destined for municipal water supplies to interrupt transmission. Considering that a high percentage of swine in many parts of the world are infected with *B. coli*, when pigs share the same space with humans (peridomestic habitat), as is the case in many parts of the less-developed world, the risk of infection is high.^{29, 30}

References

1. da Silva, R. K. M., et al., Balantidiasis in humans: A systematic review and meta-analysis. *Acta Trop* **2021**, 223, 106069.
2. Mathison, B. A.; Pritt, B. S., Medical Parasitology Taxonomy Update, 2016-2017. *J Clin Microbiol* **2019**, 57 (2).
3. Nakauchi, K., The prevalence of *Balantidium coli* infection in fifty-six mammalian species. *The Journal of veterinary medical science / the Japanese Society of Veterinary Science* **1999**, 61 (1), 63-5.
4. Nuti, M., et al., An endemic focus of balantidiasis in the Seychelles Islands. In *Abstracts of the 10th Internat Congr Trop Med Malaria* p **1980**, 13
5. Chavalittamrong, B.; Jirapinyo, P., Intestinal parasites in pediatric patients with diarrhoeal diseases in Bangkok. *The Southeast Asian journal of tropical medicine and public health* **1984**, 15 (3), 385-8.
6. Currie, A. R., Human balantidiasis. A case report. *South African journal of surgery. Suid-Afrikaanse tydskrif vir chirurgie* **1990**, 28 (1), 23-5.
7. Clyti, E., et al., [A case of coinfection by *Balantidium coli* and HIV in French Guiana]. *Bulletin de la Societe de pathologie exotique (1990)* **1998**, 91 (4), 309-11.
8. Hindsbo, O., et al., Age-dependent occurrence of the intestinal ciliate *Balantidium coli* in pigs at a

- Danish research farm. *Acta veterinaria Scandinavica* **2000**, 41 (1), 79-83.
9. da Silva Barbosa, A., et al., First molecular characterization of *Balantiodies coli* (Malmsten, 1857) isolates maintained in vitro culture and from feces of captive animals, Rio de Janeiro, Brazil. *Vet Parasitol Reg Stud Reports* **2017**, 10, 102-113.
10. Ponce-Gordo, F.; Garcia-Rodriguez, J. J., *Balantiodies coli*. *Research in veterinary science* **2021**, 135, 424-431.
11. Schuster, F. L.; Ramirez-Avila, L., Current world status of *Balantidium coli*. *Clin Microbiol Rev* **2008**, 21 (4), 626-38.
12. Vasilakopoulos, A., et al., *Balantidium coli* pneumonia in an immunocompromised patient. *Scand Dis* **2003**, 35, 144-146.
13. Malmsten, P. H., Infusorien als intestinal-Tiere beim Menschen. *Arch Pathol Anat Physiol Klin Med Virchow* **1857**, 12 302-309.
14. Chistyakova, L. V., et al., Reisolation and redescription of *Balantidium duodeni* Stein, 1867 (Litostomatea, Trichostomatia). *Parasitol Res* **2014**, 113 (11), 4207-15.
15. Wenger, R., [On differential electro-vectorcardiographic diagnosis of hypertrophy of the right ventricle]. *Atti della Societa italiana di cardiologia* **1967**, 2, 91-2.
16. Arean, V. M., et al., Balantidiasis: a review and report of cases. *Am* **1956**, 32, 1089-1117.
17. Martinez-Paloma, A., et al., Topley and Wilson's Microbiology and Microbial Infections 9th ed. 1998; p 157-177.
18. Walzer, P. D., et al., Balantidiasis outbreak in Truk. *Am J Trop Med Hyg* **1973**, 22 (1), 33-41.
19. Tempelis, C. H.; Lysenko, M. G., The production of hyaluronidase by *B coli*. *Exp Parasitol* **1957**, 6, 31-36.
20. Young, M. D., Attempts to transmit human *Balantidium coli*. *Am J Trop Med Hyg* **1950**, 30 (1), 71.
21. Ladas, S. D., et al., Invasive balantidiasis presented as chronic colitis and lung involvement. *Dig Dis Sci* **1989**, 34 (10), 1621-3.
22. Dorfman, S., et al., Balantidiasis: report of a fatal case with appendicular and pulmonary involvement. *Trans R Soc Trop Med Hyg* **1984**, 78 (6), 833-4.
23. Esteban, J. G., et al., Balantidiasis in Aymara children from the northern Bolivian Altiplano. *Am J Trop Med Hyg* **1998**, 59 (6), 922-7.
24. Dodd, L. G., *Balantidium coli* infestation as a cause of acute appendicitis. *The Journal of infectious diseases* **1991**, 163 (6), 1392.
25. Karuna, T.; Khadanga, S., A rare case of urinary balantidiasis in an elderly renal failure patient. *Trop Parasitol* **2014**, 4 (1), 47-9.
26. Castro, J., et al., Dysentery caused by *Balantidium coli*-report of two cases. *Endoscopy* **1983**, 15 (4), 272-4.
27. McHardy, I. H., et al., Detection of intestinal protozoa in the clinical laboratory. *J Clin Microbiol* **2014**, 52 (3), 712-20.
28. Ochoa, T. J.; White, A. C., Jr., Nitazoxanide for treatment of intestinal parasites in children. *Pediatr Infect Dis J* **2005**, 24 (7), 641-2.
29. Morris, R. G., et al., Prevalence of gastrointestinal parasitism in Oklahoma swine. *Am J Vet Res* **1984**, 45 (11), 2421-3.
30. Solaymani-Mohammadi, S., et al., Intestinal protozoa in wild boars (*Sus scrofa*) in western Iran. *J Wildl Dis* **2004**, 40 (4), 801-3.



Carlos Justiniano Rubeiro Chagas, M.D. (1879–1934)

Working as a physician in rural Brazil, Chagas determined that the most recent seasonal mortality rate was not due entirely to malaria, as many had died with no malarial parasites demonstrable in their bloodstream. Instead, he observed that the vinchuca bug (reduviid) had trypanosomes in their gut tract and made the association between the bugs and the infection in people by finding trypanosomes in the blood of a young infected girl. He went on to describe many of the clinical features of what was to become known as American trypanosomiasis. He named it *Trypanosoma cruzi*, after his friend and mentor, Oswaldo Cruz. Chagas went on to discover *Pneumocystis carinii*, (renamed *Pneumocystis jirovecii*) an opportunistic fungal infection, a particular risk for HIV/AIDS patients.

14. Other Protozoa of Medical Importance

Babesia spp. (Victor Babeș 1888)

Pronunciation: \bə-ˈbē-zhē-ə\

Introduction

Babesia (\bah-BEE-zhee-ah\) spp. comprise a group of genetically related intracellular protozoa that infect red blood cells.¹ They are closely related to the malarias belonging to the phylum Apicomplexa. *Babesia* spp. are all vector-borne infections transmitted by the bite of ticks. Babesiosis in humans can manifest as a mild fever or progress to a severe, life-threatening illness. There are over 100 species that infect numerous hosts, but very few are responsible for most human infections. This is a pathogen that affects not only humans but also domestic cattle. For example, *B. bigemina* occasionally infects humans but primarily infects cows, causing extensive economic loss wherever it is endemic. *B. microti* is the most common species infecting rodents and humans in the United States.² Several species that infect dogs and cats may sometimes be responsible for human infection.^{3,4} Babesiosis in humans was considered a rarity, yet 139 cases were reported in New York State between 1982 and 1993.⁵ Currently, babesiosis is as prevalent in some areas as Lyme disease. It is now classified as an emerging infection by the Centers for Disease Control (CDC) and one day may warrant a chapter of its own.⁶⁻⁸

B. divergens is the most frequently occurring species in Europe and is also considered an emerging infection in that region of the world.⁹ Many species overlap in their geographic distribution, but most have discrete ecological niches, primarily determined by their restriction to certain species of ticks.

Historical Information

In 1888, the Romanian pathologist Victor Babeș (\viktor babe\), after whom the genus is named, identified these intraerythrocytic microorganisms as causing febrile hemoglobinuria in cattle.¹⁰ A few years later, in 1893, Theobald Smith and Frederick L. Kilbourne demonstrated that ticks were the vectors of *B. bigemina*, the cause of Texas cattle fever in the Southwestern United States. This infectious agent was the first one shown to be vector-borne.¹¹ Their finding inspired others to look for additional diseases transmitted by arthropods. Shortly after that, the mosquito vectors for yellow fever and malaria were described. *Babesia* spp. were not recognized as human pathogens until 1957, when a case report was published on *B. divergens* as an infectious agent in a splenectomized herdsman.¹² In 1969, a case of babesiosis was identified in an immunocompetent 59-year-old widow living on Nantucket Island, a small island off the coast of Massachusetts in the United States.¹³ After additional cases were reported, this became known in some areas as “Nantucket fever.”

Life Cycle

Ixodes scapularis is the vector for *B. microti* in many parts of the United States (the same one that transmits Lyme disease), and *Ixodes ricinus* is the primary vector for *B. divergens* in Europe. Because *Babesia* spp. infects red cells, infections can and have been acquired by blood transfusion.¹⁴

Infection begins with the introduction of sporozoites contained within the salivary secretions of the larval tick. The sporozoites bind to glycosaminoglycans and sialoglycoproteins on erythrocytes. They enter these cells (Fig. 14.1) by inducing a deformation in the membrane, creating a parasitophorous vacu-

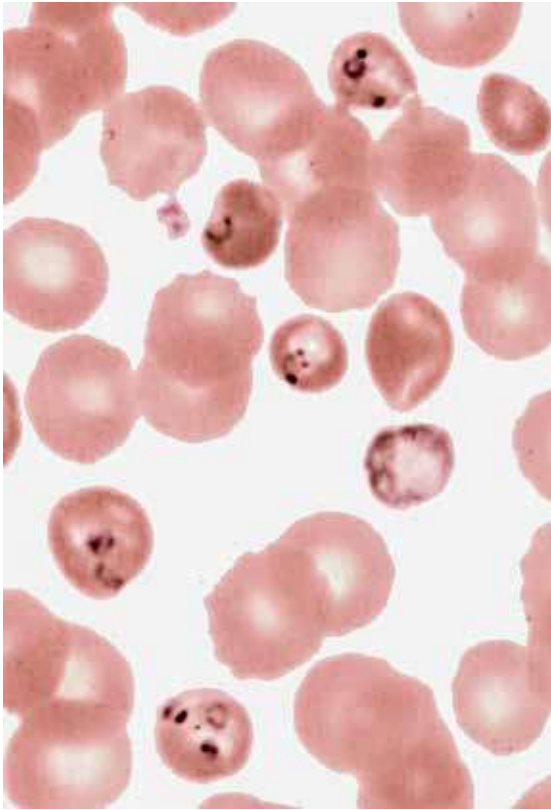


Figure 14.1. Red cells infected with various stages of *Babesia microti*.

ole.¹⁵⁻¹⁸ The sporozoite grows and develops, maturing into merozoites that divide. The parasitophorous vacuole then breaks down, leaving the organism bathed in the naked red cell cytosol. It is inferred from knowing its ecological niche that *Babesia* ingests and utilizes hemoglobin as a nutritional source. In contrast with *Plasmodium*, *Babesia* does not discard hemozoin within the red blood cells. Levels of parasitized red blood cells are usually in the range of 1–10% but can be as high as 80%, sometimes without pathological consequences.¹⁹⁻²¹ Egress from the red cells results in the rupture of host erythrocytes.

The sexual part of the life cycle takes place in the tick, where gametocytes differentiate into gametes. Reproduction is by schizogony. Gametes migrate into the hemolymph, where ookinetes develop two weeks after the vector ingested the organisms. Ookinetes migrate to the tick salivary glands, leading to infectious

haploid sporozoites.⁶ Larval ticks remain infected throughout the winter months. After developing to the nymph stage, they can transmit the infection to another mammalian host.²²

Clinical Disease

Usually, after a gradual onset of nonspecific symptoms, patients experience high fever. In the United States, infected individuals typically present in one of two ways. Immunocompetent individuals develop a self-limiting disease of mild duration, lasting 2–4 weeks.²³⁻²⁵ The self-limiting disease in immunocompetent patients is typically mild and often goes unnoticed without a visit to a physician but, in some cases, can progress to severe disease requiring hospitalization.²⁶ The second presentation is in the immunocompromised person. Those who are asplenic, have HIV/AIDS, have cancer, or are on immunosuppressive medication may have a more fulminant course. Patients with generalized myalgias often experience fever, malaise, headache, and occasionally bradycardia with lymphopenia.²⁷ Disease severity appears to be dictated by the immune status of the patient, with fatality rates over 20% in some immunosuppressed populations.²¹ Patients without spleens are at the most significant risk of dying from babesiosis.²⁸⁻³¹ Patients may also be coinfecting with other tick-borne pathogens, such as *Borrelia burgdorferi* (Lyme disease) and *Ehrlichia* spp. (ehrlichiosis), complicating the clinical picture.³²⁻³⁷ In Europe, infection due to *B. divergens* is usually more severe.^{24, 25} Intravascular hemolysis and hemoglobinuria are common and may necessitate whole-body transfusion as an emergency procedure.

In human infection with *B. microti*, the ratio of CD4⁺ T cells to CD8⁺ T cells decreases during infection, while natural killer cells, interferon- γ , TNF, interleukin-2, and inter-

leukin-6 increase. These results indicate that natural killer cells may be important in modulating infection.³⁸ B cells may also be important, as severe infections have developed in patients on B cell-depleting therapies such as the monoclonal antibody rituximab.³⁹ Patients with HIV/AIDS may develop a long-term chronic infection despite specific treatment for babesiosis.⁴⁰

Diagnosis (see Clinical Appendix)

Diagnosis is typically made by examination of Wright's or Giemsa-stained thin blood smears.^{41, 42} Intraerythrocytic and extracellular rings can be seen, and rarely, the pathognomonic finding of tetrads of merozoites appearing as a "Maltese Cross" can be visualized on a thin blood smear.⁴³ PCR is sensitive and specific for the diagnosis of babesiosis.⁴⁴ An ELISA-based test has been developed for both IgM and IgG for *B. microti*, but this test will not detect antibodies to other *Babesia* spp.^{6, 45} Guidelines from the Infectious Diseases Society of America published in 2020 recommend that the diagnosis of *Babesia* be confirmed by blood smear examination or PCR because of the ability of antibodies to persist even after the infection has been cleared.⁴⁶

Treatment (see Clinical Appendix)

Combination therapy with atovaquone/azithromycin is the treatment of choice for most cases in immunocompetent patients, while the combination of clindamycin/quinine is recommended as an alternative.^{6, 47} For severe disease, IV azithromycin combined with oral atovaquone can still be used with success and is the preferred antimicrobial combination in the 2020 guidelines from the Infectious Diseases Society of America.⁶
⁴⁶⁻⁴⁸ For some cases of severe disease, such as those requiring exchange transfusion, IV

clindamycin combined with either oral quinine or IV quinidine can be considered as an alternative treatment, but about half of the treated patients experience side effects such as tinnitus, gastrointestinal upset, and vertigo.^{48, 49} Exchange transfusion is sometimes employed when patients have levels of parasitemia >10%, are multisystem compromised, or are infected with *B. divergens*.²⁵ With proper therapy, mortality from babesiosis requiring hospitalization is low at about 1%.²⁶ In some individuals, the infection may persist, even after therapy.^{50, 51} In immunocompetent patients, treatment duration is usually 7-10 days, while in immunocompromised patients, six weeks or more may be required. Extending courses beyond 7-10 days may be necessary in cases with persistent parasitemia in patients who appear otherwise immunocompetent.⁵² Monitoring of parasitemia and clinical status is recommended.⁴⁶

Prevention and Control

Since *Babesia* spp. infects several reservoir host species, domestic and wild; avoiding environments where infection occurs is often difficult or impossible. Prevention at an individual level includes checking for nymphal stage ticks (note: this stage of *Ixodes* is quite challenging to visualize due to its small size) at the end of each trip into a wooded area and taking precautions to cover up the lower portion of pants with socks. This advice is particularly relevant for those living in the Northeastern regions of the United States, where the prevalence of *Borrelia burgdorferi* in some populations of *Ixodes* ticks is as high as 50%. DEET sprayed at the bottom of pants may also help. There are no vaccines against *Babesia* for humans, but ones for use in cattle are under development.⁵³ Burning understory in wooded, tick-infested regions may prove helpful in ecologically controlling infection in ticks.⁵⁴



Figure 14.2. Unsporulated oocyst of *C. belli*. 20 μ m.

***Cystoisospora belli* (Formerly known as *Isospora belli*)**
(Wenyon 1923)

Pronunciation: \sist-ō-ī-'sās-pə-rə\ be-lē\

Introduction

Cystoisospora belli (sist-oo-isos-po-ra//be-lee\) is an Apicomplexan intracellular parasite of humans that lives within enterocytes of the small intestine.⁵⁵ *C. belli* is a rare infection in immunocompetent individuals. In recent years, it has emerged as a severe diarrheal disease in patients who have HIV/AIDS and in other immunosuppressed individuals.^{56, 57}



Figure 14.3. Sporulated oocyst of *C. belli*. 20 μ m.

Life Cycle

Infection is initiated by ingesting the infective sporulated oocyst in fecally contaminated food or beverages. Oocysts require 1–2 days outside the host to undergo sporulation and become infectious to a new host. Direct person-to-person transmission is not thought to occur.^{58, 59} The oocyst (Figs. 14.2, 14.3) measures approximately 25 μ m by 15 μ m. Four sporozoites reside within each of the two sporocysts contained by the oocyst. Digestion of the cyst wall causes the release of the sporozoites into the lumen of the small intestine, and there they enter columnar epithelial cells. Asexual reproduction follows, leading to increased numbers of merozoites. *C. belli* infection in humans is similar to the sexual phase of *Toxoplasma gondii* in the cat. Occasionally, gametocytes may develop, resulting in the production of an oocyst. The molecular events controlling oocyst formation have yet to be elucidated. Oocysts are passed in the fecal mass unsporulated and are non-infectious. In HIV/AIDS patients, *C. belli* can invade and reproduce in other organs, such as the gallbladder, liver, spleen, and lymph nodes.^{60–62}

Pathogenesis

C. belli causes protracted, secretory diarrhea in AIDS patients with depressed CD4⁺ counts. In patients suffering from other varieties of immunosuppression, the disease resembles that induced by *Cryptosporidium*.^{63–66} In most immunocompetent patient populations, infection is either subclinical or the diarrhea is transitory. Malabsorption of fats in immunocompromised patients has been reported.⁶⁷

Clinical Disease

Patients infected with *C. belli* may experience fever, abdominal cramping, diarrhea, malaise, and weight loss. HIV/AIDS patients

may present with severe disease, including watery, non-bloody diarrhea, malabsorption syndrome with steatorrhea, foul-smelling stools, vomiting, and dehydration.⁶⁸ Patients may die from wasting associated with protracted weight loss and electrolyte imbalance.⁶⁹ Usually, symptoms resolve with specific infection treatment or rescue of the immune system with HAART.

Diagnosis (see Clinical Appendix)

C. belli, along with *Dientamoeba fragilis* and *Sarcocystis* spp., is one of the few protozoan pathogens that cause peripheral blood eosinophilia.⁷⁰⁻⁷² This can be a clue to considering this pathogen in the differential diagnosis of a patient with diarrhea. Identifying the unsporulated oocysts by microscopy is the definitive diagnostic test of choice. The oocysts can be visualized with modified acid-fast staining and show autofluorescence when illuminated with UV in the 330–380 nm wavelength range.⁵⁷ If stool examinations are unrevealing, duodenal aspirates or biopsy of small intestinal tissue can reveal the intracellular parasites.

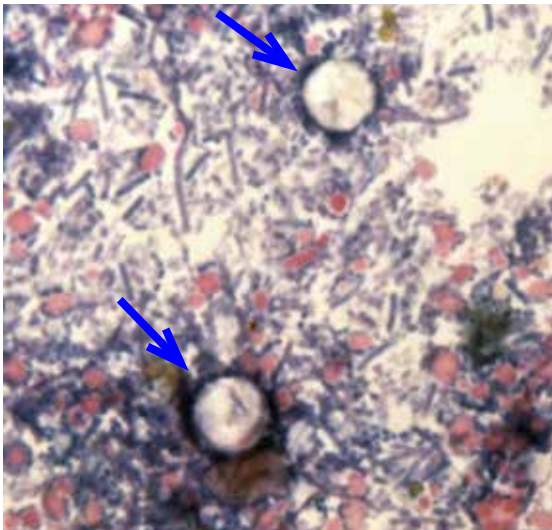


Figure 14.4. Oocyst of *C. cayetanensis*. 10 μ m.

Treatment (see Clinical Appendix)

The disease is usually self-limited in an immunocompetent host, and treatment is not always required. Fluid and electrolyte support should be addressed. If treatment is indicated, then trimethoprim-sulfamethoxazole is the therapy of choice. Its use for prevention in the HIV-infected population, along with HAART, has effectively decreased the incidence of this pathogen in many parts of the world.⁷³ Ciprofloxacin and pyrimethamine are alternative adult therapies but may be less effective options.⁷⁴ Although associated with some failures, nitazoxanide is another option with some efficacy in treating *C. belli*.^{75, 76}

Prevention and control

Avoiding fecal contamination of food and properly disposing of human feces is the best way to prevent infection.

Cyclospora cayetanensis

(Ynes R. Ortega 1991)

Pronunciation: \sī-klō-'spōr-ə\

Introduction

Cyclospora cayetanensis (\SCI-KLO-spor-ah\) causes watery diarrhea in humans and is acquired from contaminated food and water.⁷⁷⁻⁷⁹ Although this infection was virtually unknown, it has been recognized as a significant cause of diarrhea worldwide, including in the United States, where thousands of cases occur per year, with some requiring hospitalization.⁸⁰⁻⁸² In some populations, particularly in tropical regions of Peru, Brazil, and Haiti, it is endemic, usually only causing mild disease in children and adults but more severe disease in immunocompromised individuals, such as those with HIV/AIDS.^{83, 84}

In regions where this disease is endemic, epidemics tend to be seasonal, and studies conducted in Guatemala coincided with the spring raspberry harvest.⁸⁵⁻⁸⁷ In the United States, most cases are seen in returning travelers or after ingesting imported contaminated foods such as raspberries, basil, snow peas, and mesclun lettuce.^{82, 88-90} Several outbreaks in the United States have been caused by ingestion of fecally contaminated imported raspberries that may have come from Guatemala.⁹¹⁻⁹³

The life cycle can be completed in a single host species. More details about the life cycle or the role of reservoir hosts in maintaining infection in the environment are needed.⁹⁴⁻⁹⁶ Studies surveying feces from multiple animals have reported the presence of *Cyclospora* oocysts in numerous animal species, suggesting a role for reservoirs in maintaining this organism in the environment. Others report not detecting oocysts and multiple unsuccessful attempts to infect various types of animals.⁹⁷

Historical Information

In 1979, Bailey Ashford described several cases of diarrhea in Papua New Guinea with a coccidian detected in the stool.⁹⁸ A few years later, in 1986, Rosemary Soave reported several cases of diarrhea in returning travelers that may have been due to a coccidian pathogen.⁹⁹ Then, in 1991, Ynes R. Ortega characterized this organism as a new coccidian species and named this organism *Cyclospora cayetanensis* after her alma mater, the Peruvian University Cayetano Heredia in Lima, Peru.¹⁰⁰

Life Cycle

Unsporulated oocysts are released into the environment by an infected host. Oocysts require 1–2 weeks to sporulate and become infectious. Ingestion of a sporulated oocyst

begins the next round of infection (Fig. 14.4). Excystation of sporozoites occurs in the small intestine, where they attach to epithelial cells. An initial asexual stage divides and then develops into the sexual stage, producing unsporulated oocysts that are shed into the environment.⁹⁷

Clinical Disease

The clinical presentation and frequency of asymptomatic infected individuals varies in different parts of the world where *C. cayetanensis* is endemic. Infections tend to decrease in severity and duration after repeated exposures.¹⁰¹ Following the initial infection, watery diarrhea with 5–15 bowel movements per day occurs within a week or two, depending upon the initial number of ingested oocysts. In some cases, the onset of symptoms may be as long as two months after exposure.¹⁰² In immunocompetent individuals, symptoms can last up to 2–3 weeks.¹⁰³ In those with HIV/AIDS, diarrhea can be protracted.¹⁰⁴ Nausea, vomiting, anorexia, and abdominal cramping are frequent symptoms during the acute phase of the infection.⁹⁵ Less common presentations, such as biliary disease, have been recorded. Post-infectious immune-mediated complications such as Guillain-Barré syndrome and reactive arthritis have been reported.^{105, 106}

Diagnosis (see Clinical Appendix)

Diagnosis can be made by NAAT or microscopic identification of oocysts in stool samples (Fig. 14.4). *C. cayetanensis* oocysts can be seen without staining, but modified acid-fast techniques can improve the sensitivity.¹⁰⁴ PCR was initially introduced to monitor foods. Molecular testing is used clinically and included in fecal NAAT panels, such as the BioFire Film Array gastrointestinal (GI) panel.^{107, 108}

Treatment, Prevention, and Control (see Clinical Appendix)

Trimethoprim-sulfamethoxazole is the drug of choice for treating infection with *C. cayetanensis*.^{109,110} Ciprofloxacin is an alternative for patients with sulfa allergies, but this option may result in lower cure rates.¹¹¹ Nitazoxanide may also be an option for treatment.¹¹²

The source of infection is typically fecally contaminated food, so prevention and control at the community level is possible by employing good public health practices, especially in agricultural settings.

Free-living Amoeba (*Naegleria fowleri* and others) (Culbertson 1970)

Pronunciation: \nā- 'glir-ē-ə\

Introduction

Several groups of free-living amoebae cause severe disease in humans; *Naegleria fowleri* (\NAY-glir-EE-ah\), various species of *Acanthamoeba* (*A. astronyxis*, *A. culbertsoni*, *A. castellanii*, *A. polyphaga*, *A. rhysodes*, and *A. hatchetti*), and *Balamuthia mandrillaris*.¹¹³ All these species are distributed worldwide and isolated from all types of freshwater habitats and soils.

Naegleria fowleri

Pronunciation: \nā- 'glir-ē-ə\

N. fowleri, known as the brain-eating amoeba, is a robust thermophilic free-living amoeba found worldwide in warm freshwater.¹¹⁴ It thrives in standing freshwater environments like hot springs, heated swimming pools, and hot tubs. The trophozoite (Fig. 14.5) measures 20–30 μm in diameter, while its cyst

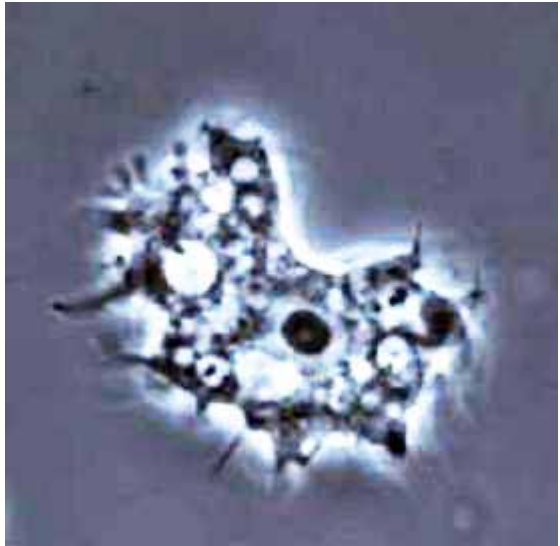


Figure 14.5. Trophozoite of *Naegleria fowleri*. 25 μm .

is smaller, measuring 8–10 μm in diameter. *N. fowleri* was abundant in the thermal spas built by a Roman legion in what is now Bath, England.¹¹⁵ Its discovery there caused the spa to be temporarily closed, and the resulting archeological dig through the sediments built up over the centuries led to fascinating glimpses into the lives of this ancient army during its occupation of that region.

Clinical Disease

N. fowleri causes a serious, often fatal, fulminating infection of the CNS, referred to as primary amoebic meningoencephalitis (PAM).^{116,117} Cases of PAM have occurred in the United States, Europe, Australia, South and Central America, and Southeast Asia, but the true burden of the disease is likely underestimated.¹¹⁸

The infection is typically acquired by swimming or bathing in warm freshwater.¹¹⁹ This environment results in the selection of an abundance of thermally-tolerant organisms, including *N. fowleri*.¹²⁰ Diving or playful splashing can force heated water containing trophozoites into the nose and through the

cribriform plate. Watersports, use of nasal irrigation, and summertime “splash pads” can also be associated with infection.¹¹⁹ *N. fowleri* invades and migrates along the base of the brain and then penetrates deeply into the cortex, producing an acute inflammatory reaction with extensive areas of lysis. Amoebae lyse through tissue, probably aided by a pore-forming protein similar to *Entamoeba histolytica*.¹²¹ Symptoms include severe frontal headache, vomiting, confusion, fever, and coma, followed by death.¹²²

Diagnosis (see Clinical Appendix)

Motile *N. fowleri* trophozoites can sometimes be isolated from CSF and visualized with microscopy. In this case, the fluid should be concentrated, a smear made, stained with Giemsa or Wright’s stain, and examined microscopically. An immunofluorescent assay, culture techniques, and multiplex PCR have been introduced to improve detection sensitivity.^{123, 124} Biopsy is another option that may reveal organisms. Metagenomic next-generation sequencing of CSF and plasma is a developing option for diagnosing *N. fowleri* and other free-living amoeba species, but it is not yet widely available.¹²⁵ Diagnosis can be delayed because PAM resembles symptoms of meningitis, a more commonly occurring clinical entity. Since death often ensues within five days after acquiring PAM, rapid diagnosis is essential. *N. fowleri* may be suspected in an otherwise healthy young adult with a recent history of contact with heated water. DNA probes specific to *N. fowleri* have been developed for its detection in water samples.¹²⁶

Treatment and Prevention (see Clinical Appendix)

Amphotericin B was the initial therapeutic agent used to treat patients, but mortal-

ity exceeded 95% despite administration.¹²⁷ In 2013, two young children in the United States were treated with miltefosine added to their treatment regimen, and both children survived.^{128, 129} Prolonged courses of multidrug therapy with Amphotericin B, rifampin, fluconazole, miltefosine, azithromycin, and steroids are used to control cerebral inflammation.¹²⁸ More data are needed to understand the optimal treatment of this disease. The CDC’s Free Living Ameba branch should be contacted to guide therapy in any suspected case of *N. fowleri*, and are available via the following phone number: 770-488-7100.

N. fowleri is ubiquitous in distribution. One study conducted in Oklahoma showed that the number of pathogenic free-living amoeba species varied throughout the seasons and were most prevalent in natural water sources (i.e., lakes and impoundments) in the spring and fall. This suggests that the organisms are generally found in benthic zones (the lowest levels of water) and only gain access to the water column during periods of lake “turn-over.”¹³⁰ Certain behaviors include diving into warm water or using sinus rinses and religious ablutions where warm water, potentially containing these amoebae, is forced up the nose. Modifying these behaviors may reduce risk, including using nasal clips, avoiding jumping or diving into warm freshwater, and using only distilled water for nasal rinses or similar purposes.

As this pathogen is ubiquitous, and the number of visits to hot tubs, spas, and natural hot springs is so high, it is a problem why this disease is so rare. Possibilities may include undiscovered host risk factors, either unique errors in metabolism or immunity, or the possibility that infections are more likely if the blood-brain barrier is somehow breached through subclinical trauma. Much more remains to be learned about this disease.

Acanthamoeba spp.

Pronunciation: \ə-ˈkanth-ə-ˈmē-bə\

Clinical Disease

Acanthamoebae (ah-KAN-thah-MEE-bah\)
are free-living amoeba associated with keratitis and granulomatous amoebic encephalitis (GAE). *Acanthamoeba* spp. infections most commonly occur in immunocompromised patients, especially those with HIV/AIDS, and may present as GAE, skin lesions, or disseminated disease.¹³¹ In immunocompetent individuals who wear contact lenses, *Acanthamoeba* may cause destructive keratitis.¹³²
¹³³ The growing number of immunocompromised individuals and the increase in contact lens wearers contribute to the rise in human cases.¹³⁴

Acanthamoebae have both a trophozoite and a cyst stage. The trophozoite and the cyst are approximately 13–23 µm in diameter.¹³⁵ The route of infection of *Acanthamoeba* spp. is most likely via the lungs or skin, resulting in multiple foci of infection.¹³⁶ Given this organism's ubiquitous nature, it is probable that almost all human beings encounter them at some point during their lives. Disease is most likely due to a particular exposure type or host susceptibility feature.¹³⁷

Acanthamoeba spp. can invade the CNS.¹³⁸ In the brain, a slowly developing, ulcerative granulomatous disease develops, characterized by diplopia, frontal headache, seizures, and, frequently, death. Patients with HIV/AIDS or other forms of immunocompromise (bone marrow transplant, malignancy) may experience overwhelming disseminated infection.^{132, 139}

Ulcerative keratitis of the eye caused by *Acanthamoeba* spp. occurs primarily in

those who routinely wash their contact lenses in unfiltered tap water.¹⁴⁰ This is now considered a rare situation, primarily due to targeted public health education programs and the availability of sterile lens cleaning solutions, but the incidence may be increasing. Infection begins with the excystation of the trophozoite under the contact lens after it is applied to the eye. Amoebae invade the cornea and begin to erode the surface, creating the sensation of burning and a perceived “gritty” consistency under the lid when the eye is closed. A ring-enhancing lesion develops, impairing vision. Partial or total blindness may ensue if left untreated.

Diagnosis (see Clinical Appendix)

A preliminary diagnosis of amebic infection may be made by microscopic identification of the amoebae in biopsy tissue, CSF, or lachrymal secretions. Definitive molecular diagnosis may be accomplished via PCR or, increasingly, metagenomic next-generation sequencing of CSF or serum.¹⁴¹ A reliable staining method employing Field's staining reagent is available.¹⁴² This test is rapid, taking only 20 minutes to carry out, and is also a valuable adjunct for field surveys.

Treatment and Prevention (see Clinical Appendix)

Although different treatments have been used for both keratitis and GAE, choosing the best treatment is problematic due to a lack of clinical experience and no controlled trials evaluating therapies.^{135, 143} Few patients with GAE have survived, regardless of treatment, particularly those with HIV/AIDS. The CDC recommends five drug regimens: miltefosine, pentamidine, sulfadiazine, flucytosine, and fluconazole.¹³¹ The CDC should be contacted to guide treatment in suspected or proven cases.

Keratitis is also challenging to treat, but some drugs show promise, particularly topical miconazole, propamidine, and Neosporin.¹⁴³ Prevention of keratitis is straightforward; use only sterile contact lens cleaning solutions. These products are easily obtained at any drug store as over-the-counter preparations. Since *Acanthamoebae* are found in countless ecological settings, it is nearly impossible to advise a method of avoiding contact with this ubiquitous group of organisms.

Balamuthia mandrillaris

(Visvesvara 1993)

Pronunciation: \bal-ə-müth-ī- ə\

Clinical Disease

Balamuthia mandrillaris (\BAL-a-moo-thee-ah\) is another free-living amoeba that can cause granulomatous amoebic encephalitis (GAE). In 1986, it was discovered in the brain of a mandrill, an Old World monkey, which died of encephalitis in the San Diego Wildlife Park.¹⁴⁴ Its name was derived from the late parasitologist William Balamuth and the mandrill from which it was first identified.¹⁴⁵ *B. mandrillaris* is found in soil. The route of initial infection is likely via the skin. Still, inhalation can also occur, resulting in an initial localized infection with the potential for spread to the brain.¹⁴⁶ Encephalitis

can occur in immunocompromised or immunocompetent individuals.¹⁴⁷ Like *Acanthamoeba*, *B. mandrillaris* has a trophozoite (15–60 µm in diameter) and a dormant cyst stage (13–30 µm in diameter).¹⁴⁵ Current investigations support the role of hematogenous spread from an initial locus of infection to distal sites such as the kidneys, lungs, adrenal glands, pancreas, thyroid, and brain. *B. mandrillaris* appears to enter the brain through the choroid plexus.¹⁴⁸ GAE can then develop. As well as GAE, patients may present with painless nodules and skin changes (more common in non-USA countries) or disseminated disease. Most patients initially present with painless nodules and skin changes at the entry site, followed by meningeal symptoms, such as fever, stiff neck, and headache that may progress to severe encephalitic manifestations with incomprehensible speech.¹⁴⁹ Uniquely among free-living amoeba infections, the transmission of *B. mandrillaris* is known to occur due to organ transplant from an infected donor, with three case clusters reported to date.^{150, 151} *B. mandrillaris* is not an infection limited to the tropics, as many cases have been recognized in the United States.¹⁵²

Diagnosis (see Clinical Appendix)

Although *B. mandrillaris* may be seen on biopsy specimens from skin lesions or distal sites such as the brain, expert knowledge is required to recognize its morphological characteristics. NAAT, CDC molecular testing, and plasma or CSF-based next-generation sequencing can definitively diagnose *B. mandrillaris* infection.¹⁵³

Treatment and Prevention (see Clinical Appendix)

This disease has been associated with high mortality once brain involvement and

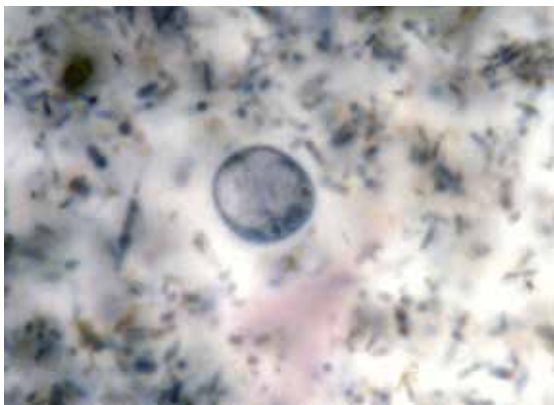


Figure 14.6. *Blastocystis hominis*. 6 µm.

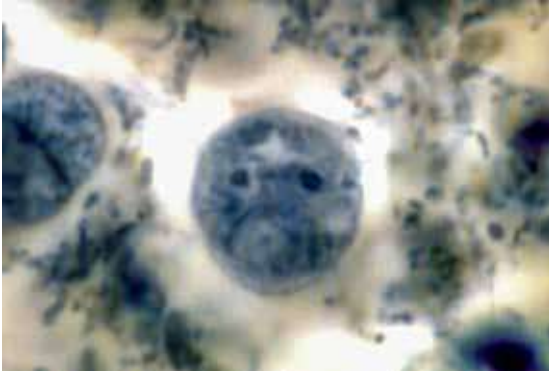


Figure 14.7. *Dientamoeba fragilis*. Note the two nuclei. 10 μ m.

GAE manifest. Initial attempts at treating patients with amphotericin were associated with patient deterioration. The use of multi-drug regimens containing multiple agents has resulted in some patients surviving and doing well despite treatment initiation after the onset of GAE. The standard of care for this disease is currently a six-drug regimen, including pentamidine, sulfadiazine, milt-efosine, flucytosine, fluconazole, and clarithromycin or azithromycin.¹⁵⁴ Other medications such as voriconazole, flucytosine, pentamidine, azithromycin, clarithromycin, trimethoprim-sulfamethoxazole, and sulfadiazine may have a role in the treatment of this pathogen.¹⁵⁵ The quinolone nitroxoline has been identified as amoebicidal and used successfully as part of a multidrug treatment regimen.¹⁵² As in other free-living amoeba infections, the CDC should be contacted to guide treatment in any suspected case.

With the limited number of cases seen and no readily identifiable host susceptibility factors in most patients, advice for prevention is currently limited.

Blastocystis hominis

(Alexieff 1911/Brumpt 1912)

Pronunciation: \Blas-to-cystis\

Blastocystis hominis (BLAS-tow-sis-TIS) is an anaerobic protozoan of uncertain taxonomic status.¹⁵⁶ *B. hominis* has been described in detail at the electron microscope level, with only two stages identified: the vacuolar stage (Fig. 14.6) and the cyst.¹⁵⁷ Other investigators have described four forms, while in reality, the extensive variation in forms is just one more aspect of this organism that makes studying its biology challenging.¹⁵⁸ Division is by binary fission. No sexual part of its life cycle has been documented. Infection is presumed via the fecal-oral route, and the cyst stage may be required for transmission. The cyst is small, measuring 2–5 μ m in diameter, and is protected by a multilayer cyst wall.¹⁵⁹ It can be grown in axenic culture, permitting studies on its biochemical, genetic, and biological properties.^{160, 161}

B. hominis is a common finding on routine stool examinations worldwide, even in asymptomatic individuals.¹⁶² This organism is also frequently encountered with other more clinically defined pathogens, and this fact alone has made deciding on its status as a pathogen, based on its epidemiology, nearly impossible. Nonetheless, it is frequently associated with gastrointestinal (GI) symptoms. Several cases have been described that defy any interpretation other than illness caused by *B. hominis*, based upon an extensive negative laboratory finding regarding all other known pathogens of the GI tract. In one case, gastroenteritis accompanied by diarrhea and hypoalbuminemia in the absence of all other pathogens was reported.¹⁶³

Many genetically distinct strains of *B. hominis* have been characterized, so some variants may be pathogenic while others are not.^{164, 165} This could explain the high degree of variability in its clinical presentation.^{166, 167} Patients with HIV/AIDS do not have an increased prevalence of *B. hominis* infection nor appear to be any more affected by its presence than the immunocompetent population. Exceptions have been reported, in which patients were symptomatic with diarrhea and were treated successfully when only *B. hominis* was found on microscopy.¹⁶⁸ People of advanced age may represent an exception under certain as-yet-undefined conditions.¹⁶⁹

Diagnosis is generally made by detecting organisms on stained smears or wet mounts of stool specimens, and PCR has been developed.¹⁷⁰ Treatment of heavily infected individuals with metronidazole, a proven antimicrobial agent against most anaerobes, was effective in eradicating *B. hominis* and improving symptoms in a high percentage of treated patients.¹⁷¹ Paromomycin is another therapy some investigators suggested as an alternative first-line therapy.¹⁷¹ Trimethoprim-sulfamethoxazole has shown efficacy in treating symptomatic patients and achieved high rates of *B. hominis* eradication from stool.¹⁷² Nitazoxanide shows promise as an effective alternative therapeutic approach. Its impact on other intestinal pathogens has confounded studies.¹⁶⁸

Dientamoeba fragilis (Jepps and Dobell 1918)

Pronunciation: \dī-ent-ə-'mē-bə\

Dientamoeba fragilis (\DYE-ent-ah-mee-bah\) is taxonomically related to *Histomonas* spp., a flagellated protozoan, but it has the morphology of an amoeba.^{173, 174} Each trophozoite (Fig. 14.7) has two nuclei. For many years, investigators had been unable to identify a cyst form, but cysts were finally identified in a mouse model and human feces.¹⁷⁵ Despite many years of controversy regarding whether or not this organism was pathogenic, it is now generally agreed that *D. fragilis* is responsible for causing a related series of gastroenteritis-like symptoms, including diarrhea and nausea with a duration often of more than two weeks.¹⁷⁶ *D. fragilis* is similar to *Cystoisospora belli* in that peripheral eosinophilia can accompany infection, and patients may develop a form of eosinophilic colitis.^{70, 177}

Diagnosis is either by direct examination of stool by microscopy or PCR. However, since trophozoites are fragile and not easily detectable on wet mounts, fixed, stained stool samples are more sensitive.¹⁷⁸

Many drugs have been shown to have some efficacy in treating infections.¹⁷⁴ Although large randomized clinical trials have yet to be carried out, metronidazole or paromomycin are recommended.^{179, 180} Nitazoxanide, tetracycline, and iodoquinol are potential options for treating *D. fragilis* based on *in vitro* susceptibility testing.¹⁸¹

References

1. Beugnet, F.; Moreau, Y., Babesiosis. *Rev Sci Tech* **2015**, 34 (2), 627-39.
2. Eskow, E. S., et al., Southern extension of the range of human babesiosis in the eastern United States. *Journal of clinical microbiology* **1999**, 37 (6), 2051-2.
3. Herwaldt, B. L., et al., Transfusion-transmitted babesiosis in Washington State: first reported case caused by a WA1-type parasite. *J Infect Dis* **1997**, 175 (5), 1259-62.
4. Telford, S. R., et al., Topley and Wilson's Microbiology and Microbial Infections. 1998; p 349-359.
5. White, D. J., et al., Human babesiosis in New York State: Review of 139 hospitalized cases and analysis of prognostic factors. *Archives of internal medicine* **1998**, 158 (19), 2149-54.
6. Vannier, E.; Krause, P. J., Human babesiosis. *N Engl J Med* **2012**, 366 (25), 2397-407.
7. Karshima, S. N., et al., Global meta-analysis on Babesia infections in human population: prevalence, distribution and species diversity. *Pathog Glob Health* **2022**, 116 (4), 220-235.
8. Swanson, M., et al., Trends in Reported Babesiosis Cases - United States, 2011-2019. *MMWR Morb Mortal Wkly Rep* **2023**, 72 (11), 273-277.
9. Gray, J. S., *Babesia* sp.: emerging intracellular parasites in Europe. *Polish journal of microbiology / Polskie Towarzystwo Mikrobiologow = The Polish Society of Microbiologists* **2004**, 53 Suppl, 55-60.
10. Babes, V., Sur l'hémogloburie bacterienne de boeuf. *C R Acad Sci* **1888**, 107, 692-4.
11. Smith, T.; Kilbourne, F. L., Investigations into the Nature, Causation, and Prevention of Texas or Southern Cattle Fever. **1893**.
12. Skrabalo, Z.; Deanovic, Z., Piroplasmosis in man; report of a case. *Doc Med Geogr Trop* **1957**, 9 (1), 11-6.
13. Western, K. A., et al., Babesiosis in a Massachusetts resident. *N Engl J Med* **1970**, 283 (16), 854-6.
14. Leiby, D. A.; Gill, J. E., Transfusion-transmitted tick-borne infections: a cornucopia of threats. *Transfusion medicine reviews* **2004**, 18 (4), 293-306.
15. Rudzinska, M. A., et al., Morphological aspects of host-cell-parasite relationships in babesiosis. 1981; p 87-141.
16. Okubo, K., et al., Calcium-ions are involved in erythrocyte invasion by equine *Babesia* parasites. *Parasitology* **2006**, 133 (Pt 3), 289-94.
17. Yokoyama, N., et al., Erythrocyte invasion by *Babesia* parasites: current advances in the elucidation of the molecular interactions between the protozoan ligands and host receptors in the invasion stage. *Vet Parasitol* **2006**, 138 (1-2), 22-32.
18. Lobo, C. A., et al., *Babesia* and red cell invasion. *Curr Opin Hematol* **2012**, 19 (3), 170-5.
19. Christianson, D.; Pollack, R. J., Persistent parasitemia after acute babesiosis. *NEJM* **1998**, 339 160-5.
20. Shih, C. M., et al., Human babesiosis in Taiwan: asymptomatic infection with a *Babesia* microti-like organism in a Taiwanese woman. *Journal of clinical microbiology* **1997**, 35 (2), 450-4.
21. Hatcher, J. C., et al., Severe babesiosis in Long Island: review of 34 cases and their complications. *Clin Infect Dis* **2001**, 32 (8), 1117-25.
22. Piesman, J., et al., Seasonal variation of transmission risk of Lyme disease and human babesiosis. *American journal of epidemiology* **1987**, 126 (6), 1187-9.
23. Boustani, M. R.; Gelfand, J. A., Babesiosis. *Clin Inf Dis* **1996**, 22 (4), 611-5.
24. Gorenflot, A., et al., Human babesiosis. *Annals of tropical medicine and parasitology* **1998**, 92 (4), 489-501.
25. Uguen, C., et al., [Human babesiosis in 1997]. *La Revue de medecine interne / fondee ... par la Societe nationale francaise de medecine interne* **1997**, 18 (12), 945-51.
26. Bloch, E. M., et al., Epidemiology of Hospitalized Patients with Babesiosis, United States, 2010-2016. *Emerg Infect Dis* **2022**, 28 (2), 354-62.
27. Kim, N., et al., Relative bradycardia and lymphopenia in patients with babesiosis. *Clin Inf Dis* **1998**, 26 (5), 1218-9.
28. Herwaldt, B., et al., A fatal case of babesiosis in Missouri: identification of another piroplasm

- that infects humans. *Annals of internal medicine* **1996**, 124 (7), 643-50.
29. Slovit, D. P., et al., Babesiosis and hemophagocytic syndrome in an asplenic renal transplant recipient. *Transplantation* **1996**, 62 (4), 537-9.
 30. Bonoan, J. T., et al., Life-threatening babesiosis in an asplenic patient treated with exchange transfusion, azithromycin, and atovaquone. *Heart & lung : the journal of critical care* **1998**, 27 (6), 424-8.
 31. Hohenschild, S., [Babesiosis--a dangerous infection for splenectomized children and adults]. *Klinische Padiatrie* **1999**, 211 (3), 137-40.
 32. Persing, D. H., The cold zone: a curious convergence of tick-transmitted diseases. *Clin Infect Dis* **25 Suppl** **1997**, 1, S35-42.
 33. Hilton, E., et al., Seroprevalence and seroconversion for tick-borne diseases in a high-risk population in the northeast United States. *The American journal of medicine* **1999**, 106 (4), 404-9.
 34. Krause, P. J., et al., Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness. *JAMA* **1996**, 275 (21), 1657-60.
 35. Mitchell, P. D., et al., Immunoserologic evidence of coinfection with *Borrelia burgdorferi*, *Babesia microti*, and human granulocytic *Ehrlichia* species in residents of Wisconsin and Minnesota. *Journal of clinical microbiology* **1996**, 34 (3), 724-7.
 36. Magnarelli, L. A., et al., Human exposure to a granulocytic *Ehrlichia* and other tick-borne agents in Connecticut. *Journal of clinical microbiology* **1998**, 36 (10), 2823-7.
 37. dos Santos, C. C.; Kain, K. C., Two tick-borne diseases in one: a case report of concurrent babesiosis and Lyme disease in Ontario. *CMAJ* **1999**, 160 (13), 1851-3.
 38. Shaio, M. F.; Lin, P. R., A case study of cytokine profiles in acute human babesiosis. *Am J Trop Med Hyg* **1998**, 58 (3), 335-7.
 39. Kelesidis, T., et al., Does rituximab increase the incidence of infectious complications? A narrative review. *Int J Infect Dis* **2011**, 15 (1), e2-16.
 40. Falagas, M. E.; Klempner, M. S., Babesiosis in patients with AIDS: a chronic infection presenting as fever of unknown origin. *Clin Inf Dis* **1996**, 22 (5), 809-12.
 41. Krause, P. J., Babesiosis diagnosis and treatment. *Vector borne and zoonotic diseases (Larchmont, N.Y.)* **2003**, 3 (1), 45-51.
 42. Homer, M. J., et al., Babesiosis. *Clinical microbiology reviews* **2000**, 13 (3), 451-69.
 43. Conrad, P. A., et al., Description of *Babesia duncani* n.sp. (Apicomplexa: Babesiidae) from humans and its differentiation from other piroplasms. *Int J Parasitol* **2006**, 36 (7), 779-89.
 44. Teal, A. E., et al., A new real-time PCR assay for improved detection of the parasite *Babesia microti*. *J Clin Microbiol* **2012**, 50 (3), 903-8.
 45. Loa, C. C., et al., Serological diagnosis of human babesiosis by IgG enzyme-linked immunosorbent assay. *Current microbiology* **2004**, 49 (6), 385-9.
 46. Krause, P. J., et al., Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA): 2020 Guideline on Diagnosis and Management of Babesiosis. *Clin Infect Dis* **2021**, 72 (2), 185-189.
 47. Shaio, M. F.; Yang, K. D., Response of babesiosis to a combined regimen of quinine and azithromycin. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1997**, 91 (2), 214-5.
 48. Krause, P. J., et al., Atovaquone and azithromycin for the treatment of babesiosis. *N Engl J Med* **2000**, 343 (20), 1454-8.
 49. Kletsova, E. A., et al., Babesiosis in Long Island: review of 62 cases focusing on treatment with azithromycin and atovaquone. *Ann Clin Microbiol Antimicrob* **2017**, 16 (1), 26.
 50. Bloch, E. M., et al., Persistence of *Babesia microti* Infection in Humans. *Pathogens* **2019**, 8 (3).
 51. Krause, P. J., et al., Persistent parasitemia after acute babesiosis. *N Engl J Med* **1998**, 339 (3), 160-5.
 52. Ivancich, M., et al., Trust the Process: Prolonged *Babesia* Parasitemia in an Elderly Man with Asplenia from the American Midwest. *Am J Case Rep* **2022**, 23, e936326.
 53. Beniwal, R. P., et al., An immunisation trial with in vitro produced *Babesia bigemina*

- exoantigens. *Tropical animal health and production* **1997**, 29 (4 Suppl), 124S-126S.
54. Stafford, K. C., et al., Impact of controlled burns on the abundance of *Ixodes scapularis* (Acari: Ixodidae). *Journal of medical entomology* **1998**, 35 (4), 510-3.
 55. Wenyon, C. M., Coccidiosis of cats and dogs and the status of the *Isospora* of man. *Ann Trop Med Parasitol* **1923**, 17, 231-39.
 56. Joshi, M., et al., Parasitic diarrhoea in patients with AIDS. *The National medical journal of India* **2002**, 15 (2), 72-4.
 57. Curry, A.; Smith, H. V., Emerging pathogens: *Isospora*, *Cyclospora* and microsporidia. *Parasitology* **1998**, 117 Suppl, S143-59.
 58. Jongwutiwes, S., et al., Morphologic and molecular characterization of *Isospora belli* oocysts from patients in Thailand. *Am J Trop Med Hyg* **2007**, 77 (1), 107-12.
 59. Ryan, E. T., et al., Case records of the Massachusetts General Hospital. Case 38-2011. A 34-year-old man with diarrhea and weakness. *N Engl J Med* **2011**, 365 (24), 2306-16.
 60. Benator, D. A., et al., *Isospora belli* infection associated with acalculous cholecystitis in a patient with AIDS. *Annals of internal medicine* **1994**, 121 (9), 663-4.
 61. Michiels, J. F., et al., Intestinal and extraintestinal *Isospora belli* infection in an AIDS patient. A second case report. *Pathology, research and practice* **1994**, 190 (11), 1089-93; discussion 1094.
 62. Restrepo, C., et al., Disseminated extraintestinal isosporiasis in a patient with acquired immune deficiency syndrome. *American journal of clinical pathology* **1987**, 87 (4), 536-42.
 63. Heyworth, M. F., Parasitic diseases in immunocompromised hosts. Cryptosporidiosis, isosporiasis, and strongyloidiasis. *Gastroenterology clinics of North America* **1996**, 25 (3), 691-707.
 64. Sahu, A. R., et al., *Isospora* induced diarrhea in a pemphigus vulgaris patient. *Indian J Dermatol Venereol Leprol* **2014**, 80 (4), 342-3.
 65. Kim, M. J., et al., *Isospora belli* Infection with Chronic Diarrhea in an Alcoholic Patient. *Korean J Parasitol* **2013**, 51 (2), 207-12.
 66. Stein, J., et al., An unusual complication in ulcerative colitis during treatment with azathioprine and infliximab: *Isospora belli* as 'Casus belli'. *BMJ Case Rep* **2013**, 2013.
 67. Kitsukawa, K., et al., [An autopsy case of T-cell lymphoma associated with disseminated varicella and malabsorption syndrome due to *Isospora belli* infection (author's transl)]. [*Rinsho ketsueki*] *The Japanese journal of clinical hematology* **1981**, 22 (2), 258-65.
 68. DeHovitz, J. A., et al., Clinical manifestations and therapy of *Isospora belli* infection in patients with the acquired immunodeficiency syndrome. *N Engl J Med* **1986**, 315 (2), 87-90.
 69. Pape, J. W.; Johnson, W. D., *Isospora belli* infections. *Progress in clinical parasitology* **1991**, 2, 119-27.
 70. Gray, T. J., et al., *Dientamoeba fragilis*: a family cluster of disease associated with marked peripheral eosinophilia. *Clin Infect Dis* **2013**, 57 (6), 845-8.
 71. Navaneethan, U., et al., *Isospora belli* superinfection in a patient with eosinophilic gastroenteritis—a diagnostic challenge. *J Crohns Colitis* **2012**, 6 (2), 236-9.
 72. Fayer, R., et al., Human infections with *Sarcocystis* species. *Clin Microbiol Rev* **2015**, 28 (2), 295-311.
 73. Mohanty, I., et al., Prevalence of isosporiasis in relation to CD4 cell counts among HIV-infected patients with diarrhea in Odisha, India. *Adv Biomed Res* **2013**, 2, 61.
 74. St. Georgiev, V., Opportunistic infections: treatment and developmental therapeutics of cryptosporidiosis and isosporiasis. *Drug Develop Res* **1993**, 28, 445-59.
 75. Bialek, R., et al., Case report: Nitazoxanide treatment failure in chronic isosporiasis. *Am J Trop Med Hyg* **2001**, 65 (2), 94-5.
 76. Doumbo, O., et al., Nitazoxanide in the treatment of cryptosporidial diarrhea and other intestinal parasitic infections associated with acquired immunodeficiency syndrome in tropical Africa. *Am J Trop Med Hyg* **1997**, 56 (6), 637-9.
 77. Ortega, Y. R., et al., *Cyclospora cayetanensis*. *Advances in parasitology* **1998**, 40, 399-418.
 78. Rose, J. B.; Slifko, T. R., *Giardia*, *Cryptosporidium*, and *Cyclospora* and their impact on foods: a review. *Journal of food protection* **1999**, 62 (9), 1059-70.
 79. Mansfield, L. S.; Gajadhar, A. A., *Cyclospora cayetanensis*, a food- and waterborne coccidian

- parasite. *Vet Parasitol* **2004**, 126 (1-2), 73-90.
80. Sterling, C. R.; Ortega, Y. R., *Cyclospora*: an enigma worth unraveling. *Emerging infectious diseases* **1999**, 5 (1), 48-53.
81. Mead, P. S., et al., Food-related illness and death in the United States. *Emerg Infect Dis* **1999**, 5 (5), 607-25.
82. Rehme, P., Notes from the Field: Doubling of Cyclosporiasis Cases Partially Attributable to a Salad Kit - Florida, 2021-2022. *MMWR Morb Mortal Wkly Rep* **2023**, 72 (27), 751-752.
83. Eberhard, M. L., et al., *Cyclospora cayetanensis* infections in Haiti: a common occurrence in the absence of watery diarrhea. *Am J Trop Med Hyg* **1999**, 60 (4), 584-6.
84. Pape, J. W., et al., *Cyclospora* infection in adults infected with HIV. Clinical manifestations, treatment, and prophylaxis. *Annals of internal medicine* **1994**, 121 (9), 654-7.
85. Bern, C., et al., The contrasting epidemiology of *Cyclospora* and *Cryptosporidium* among outpatients in Guatemala. *Am J Trop Med Hyg* **2000**, 63 (5-6), 231-5.
86. Bern, C., et al., *Cyclospora* in Guatemala: further considerations. *J Clin Microbiol* **2002**, 40 (2), 731-2.
87. Bern, C., et al., Epidemiologic studies of *Cyclospora cayetanensis* in Guatemala. *Emerg Infect Dis* **1999**, 5 (6), 766-74.
88. Chalmers, R. M., et al., Foodborne outbreaks of cyclosporiasis have arisen in North America. Is the United Kingdom at risk? *Commun Dis Public Health* **2000**, 3 (1), 50-5.
89. Manuel, D., et al., An outbreak of cyclosporiasis in 1996 associated with consumption of fresh berries- Ontario. *Can J Infect Dis* **2000**, 11 (2), 86-92.
90. Centers for Disease, C.; Prevention, Outbreak of cyclosporiasis associated with snow peas-- Pennsylvania, 2004. *MMWR Morb Mortal Wkly Rep* **2004**, 53 (37), 876-8.
91. Caceres, V. M., et al., A foodborne outbreak of cyclosporiasis caused by imported raspberries. *Pract* **1998**, 47 231-4.
92. Koumans, E. H., et al., An outbreak of cyclosporiasis in Florida in 1995: a harbinger of multistate outbreaks in 1996 and 1997. *Am J Trop Med Hyg* **1998**, 59 (2), 235-42.
93. Herwaldt, B. L.; Beach, M. J., The return of *Cyclospora* in 1997: another outbreak of cyclosporiasis in North America associated with imported raspberries. Cyclospora Working Group. *Annals of internal medicine* **1999**, 130 (3), 210-20.
94. Shields, J. M.; Olson, B. H., *Cyclospora cayetanensis*: a review of an emerging parasitic coccidian. *International journal for parasitology* **2003**, 33 (4), 371-91.
95. Ortega, Y. R., et al., Pathologic and clinical findings in patients with cyclosporiasis and a description of intracellular parasite life-cycle stages. *J Infect Dis* **1997**, 176 (6), 1584-9.
96. Smith, H. V., et al., *Cyclospora* in non-human primates in Gombe, Tanzania. *The Veterinary record* **1996**, 138 (21), 528.
97. Ortega, Y. R.; Sanchez, R., Update on *Cyclospora cayetanensis*, a food-borne and waterborne parasite. *Clin Microbiol Rev* **2010**, 23 (1), 218-34.
98. Ashford, R. W., Occurrence of an undescribed coccidian in man in Papua New Guinea. *Ann Trop Med Parasitol* **1979**, 73 (5), 497-500.
99. Soave, R.; Armstrong, D., *Cryptosporidium* and cryptosporidiosis. *Rev Infect Dis* **1986**, 8 (6), 1012-23.
100. Ortega, Y. R., et al., *Cyclospora* species--a new protozoan pathogen of humans. *N Engl J Med* **1993**, 328 (18), 1308-12.
101. Connor, B. A., et al., Cyclosporiasis: clinical and histopathologic correlates. *Clin Infect Dis* **1999**, 28 (6), 1216-22.
102. Shlim, D. R., et al., An alga-like organism associated with an outbreak of prolonged diarrhea among foreigners in Nepal. *Am J Trop Med Hyg* **1991**, 45 (3), 383-9.
103. Fleming, C. A., et al., A foodborne outbreak of *Cyclospora cayetanensis* at a wedding: clinical features and risk factors for illness. *Arch Intern Med* **1998**, 158 (10), 1121-5.
104. Blanshard, C., et al., Cryptosporidiosis in HIV-seropositive patients. *Q J Med* **1992**, 85 (307-308), 813-23.
105. Richardson, R. F., et al., Guillain-Barre syndrome after *Cyclospora* infection. *Muscle Nerve*

- 1998, 21 (5), 669-71.
106. Connor, B. A., et al., Reiter syndrome following protracted symptoms of *Cyclospora* infection. *Emerg Infect Dis* **2001**, 7 (3), 453-4.
 107. Jinneman, K. C., et al., An oligonucleotide-ligation assay for the differentiation between *Cyclospora* and *Eimeria* spp polymerase chain reaction amplification products. *Prot* 62, 682-5.
 108. Buss, S. N., et al., Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. *J Clin Microbiol* **2015**, 53 (3), 915-25.
 109. Madico, G., et al., Treatment of *Cyclospora* infections with co-trimoxazole. *Lancet* **1993**, 342 (8863), 122-3.
 110. Hoge, C. W., et al., Placebo-controlled trial of co-trimoxazole for *Cyclospora* infections among travellers and foreign residents in Nepal. *Lancet* **1995**, 345 (8951), 691-3.
 111. Verdier, R. I., et al., Trimethoprim-sulfamethoxazole compared with ciprofloxacin for treatment and prophylaxis of *Isospora belli* and *Cyclospora cayetanensis* infection in HIV-infected patients. A randomized, controlled trial. *Ann Intern Med* **2000**, 132 (11), 885-8.
 112. Zimmer, S. M., et al., Efficacy of nitazoxanide for cyclosporiasis in patients with sulfa allergy. *Clin Infect Dis* **2007**, 44 (3), 466-7.
 113. Schuster, F. L.; Visvesvara, G. S., Amebae and ciliated protozoa as causal agents of waterborne zoonotic disease. *Vet Parasitol* **2004**, 126 (1-2), 91-120.
 114. Baig, A. M.; Khan, N. A., Tackling infection owing to brain-eating amoeba. *Acta Trop* **2015**, 142, 86-8.
 115. Kilvington, S.; Beeching, J., Identification and epidemiological typing of *Naegleria fowleri* with DNA probes. *Applied and environmental microbiology* **1995**, 61 (6), 2071-8.
 116. Rodriguez, R., et al., Infection del sistema nervioso central por amebas de vida libre: comunicacion de tres nuevos casos venezolanos. *Rev Neurologia* **1998**, 26, 1005-8.
 117. Okuda, D. T., et al., *Naegleria fowleri* hemorrhagic meningoencephalitis: report of two fatalities in children. *Journal of child neurology* **2004**, 19 (3), 231-3.
 118. Ahmad Zamzuri, M. I., et al., Systematic Review of Brain-Eating Amoeba: A Decade Update. *Int J Environ Res Public Health* **2023**, 20 (4).
 119. Gharpure, R., et al., Epidemiology and Clinical Characteristics of Primary Amebic Meningoencephalitis Caused by *Naegleria fowleri*: A Global Review. *Clin Infect Dis* **2021**, 73 (1), e19-e27.
 120. Visvesvara, G. S.; Stehr-Green, J. K., Epidemiology of free-living ameba infections. *The Journal of protozoology* **1990**, 37 (4), 25S-33S.
 121. Herbst, R., et al., Antimicrobial and pore-forming peptides of free-living and potentially highly pathogenic *Naegleria fowleri* are released from the same precursor molecule. *The Journal of biological chemistry* **2004**, 279 (25), 25955-8.
 122. Martinez, A. J., Free-living amoebic meningoencephalitis: comparative study. *NeurologiaNeurocirugiaPsiquiatria* 18 Suppl 391 **1977**, 401, 2-3.
 123. Visvesvara, G. S., et al., Production of monoclonal antibodies to *Naegleria fowleri*, agent of primary amebic meningoencephalitis. *J Clin Microbiol* **1987**, 25 (9), 1629-34.
 124. Qvarnstrom, Y., et al., Multiplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*. *J Clin Microbiol* **2006**, 44 (10), 3589-95.
 125. Wang, Q., et al., A case of *Naegleria fowleri* related primary amoebic meningoencephalitis in China diagnosed by next-generation sequencing. *BMC Infect Dis* **2018**, 18 (1), 349.
 126. Behets, J., et al., Detection of *Naegleria* spp. and *Naegleria fowleri*: a comparison of flagellation tests, ELISA and PCR. *Water science and technology : a journal of the International Association on Water Pollution Research* **2003**, 47 (3), 117-22.
 127. Schuster, F. L.; Visvesvara, G. S., Opportunistic amoebae: challenges in prophylaxis and treatment. *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy* **2004**, 7 (1), 41-51.
 128. Linam, W. M., et al., Successful treatment of an adolescent with *Naegleria fowleri* primary amebic meningoencephalitis. *Pediatrics* **2015**, 135 (3), e744-8.

129. Capewell, L. G., et al., Diagnosis, Clinical Course, and Treatment of Primary Amoebic Meningoencephalitis in the United States, 1937-2013. *J Pediatric Infect Dis Soc* **2015**, 4 (4), e68-75.
130. John, D. T.; Howard, M. J., Seasonal distribution of pathogenic free-living amebae in Oklahoma waters. *Parasitology research* **1995**, 81 (3), 193-201.
131. Haston, J. C., et al., The Epidemiology and Clinical Features of Non-Keratitis Acanthamoeba Infections in the United States, 1956-2020. *Open Forum Infect Dis* **2023**, 10 (1), ofac682.
132. Paltiel, M., et al., Disseminated cutaneous acanthamebiasis: a case report and review of the literature. *Cutis* **2004**, 73 (4), 241-8.
133. Marciano-Cabral, F.; Cabral, G., *Acanthamoeba* spp. as agents of disease in humans. *Clinical microbiology reviews* **2003**, 16 (2), 273-307.
134. Khan, N. A., *Acanthamoeba*: biology and increasing importance in human health. *FEMS Microbiol Rev* **2006**, 30 (4), 564-95.
135. Siddiqui, R.; Khan, N. A., Biology and pathogenesis of *Acanthamoeba*. *Parasit Vectors* **2012**, 5, 6.
136. Martinez, A. J., Infection of the central nervous system due to *Acanthamoeba*. *Rev of Infect Dis* **13 Suppl** **1991**, 5, S399-402.
137. Brindley, N., et al., *Acanthamoeba castellanii*: high antibody prevalence in racially and ethnically diverse populations. *Exp Parasitol* **2009**, 121 (3), 254-6.
138. Koide, J., et al., Granulomatous amoebic encephalitis caused by *Acanthamoeba* in a patient with systemic lupus erythematosus. *Clinical rheumatology* **1998**, 17 (4), 329-32.
139. Sison, J. P., et al., Disseminated *Acanthamoeba* infection in patients with AIDS: case reports and review. *Clin Inf Dis* **1995**, 20 (5), 1207-16.
140. Auran, J. D., et al., *Acanthamoeba* keratitis. A review of the literature. *Cornea* **1987**, 6 (1), 2-26.
141. Goldschmidt, P., et al., New tool for the simultaneous detection of 10 different genotypes of *Acanthamoeba* available from the American Type Culture Collection. *Br J Ophthalmol* **2009**, 93 (8), 1096-100.
142. Pirehma, M., et al., Field's stain--a rapid staining method for *Acanthamoeba* spp. *Parasitology research* **1999**, 85 (10), 791-3.
143. Seal, D., Treatment of *Acanthamoeba* keratitis. *Expert review of anti-infective therapy* **2003**, 1 (2), 205-8.
144. Schuster, F. L., et al., Environmental isolation of *Balamuthia mandrillaris* associated with a case of amebic encephalitis. *J Clin Microbiol* **2003**, 41 (7), 3175-80.
145. Matin, A., et al., Increasing importance of *Balamuthia mandrillaris*. *Clin Microbiol Rev* **2008**, 21 (3), 435-48.
146. Siddiqui, R.; Khan, N. A., *Balamuthia* amoebic encephalitis: an emerging disease with fatal consequences. *Microb Pathog* **2008**, 44 (2), 89-97.
147. Cope, J. R., et al., The Epidemiology and Clinical Features of *Balamuthia mandrillaris* Disease in the United States, 1974-2016. *Clin Infect Dis* **2019**, 68 (11), 1815-1822.
148. Jayasekera, S., et al., Post-mortem culture of *Balamuthia mandrillaris* from the brain and cerebrospinal fluid of a case of granulomatous amoebic meningoencephalitis, using human brain microvascular endothelial cells. *J Med Microbiol* **2004**, 53 (Pt 10), 1007-12.
149. Martinez, A. J.; Visvesvara, G. S., *Balamuthia mandrillaris* infection. *J Med Microbiol* **2001**, 50 (3), 205-7.
150. Gupte, A. A., et al., Transmission of *Balamuthia mandrillaris* through solid organ transplantation: utility of organ recipient serology to guide clinical management. *Am J Transplant* **2014**, 14 (6), 1417-24.
151. Farnon, E. C., et al., Transmission of *Balamuthia mandrillaris* by Organ Transplantation. *Clin Infect Dis* **2016**, 63 (7), 878-888.
152. Spottiswoode, N., et al., Successful Treatment of *Balamuthia mandrillaris* Granulomatous Amebic Encephalitis with Nitroloxline. *Emerg Infect Dis* **2023**, 29 (1), 197-201.
153. Kalyatanda, G., et al., Rapid, Noninvasive Diagnosis of *Balamuthia mandrillaris* Encephalitis by a Plasma-Based Next-Generation Sequencing Test. *Open Forum Infect Dis* **2020**, 7 (7), ofaa189.

154. Deetz, T. R., et al., Successful treatment of Balamuthia amoebic encephalitis: presentation of 2 cases. *Clin Infect Dis* **2003**, 37 (10), 1304-12.
155. Parija, S. C., et al., Management of granulomatous amebic encephalitis: Laboratory diagnosis and treatment. *Trop Parasitol* **2015**, 5 (1), 23-8.
156. Nasirudeen, A. M. A.; Tan, K. S. W., Isolation and characterization of the mitochondrion-like organelle from *Blastocystis hominis*. *Journal of microbiological methods* **2004**, 58 (1), 101-9.
157. Windsor, J. J., et al., Multiple reproductive processes in *Blastocystis hominis*. *Trends in parasitology* **2003**, 19 (7), 289-90; author reply 291.
158. Tan, K. S., New insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clin Microbiol Rev* **2008**, 21 (4), 639-65.
159. Chen, X. Q., et al., In vitro encystation and excystation of *Blastocystis ratti*. *Parasitology* **1999**, 118 (Pt 2), 151-60.
160. Zaman, V., et al., Postcystic development of *Blastocystis*. *Parasitol Res* **1999**, 27 (8), 941-5.
161. Carbajal, J. A.; Castillo, L., del Karyotypic diversity among *Blastocystis hominis* isolates. *Parasitol Res* **1999**, 85, 437-40.
162. Aguiar, J. I., et al., Intestinal protozoa and helminths among Terena Indians in the State of Mato Grosso do Sul: high prevalence of *Blastocystis hominis*. *Rev Soc Bras Med Trop* **2007**, 40 (6), 631-4.
163. Nassir, E., et al., *Blastocystis hominis* as a cause of hypoalbuminemia and anasarca. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* **2004**, 23 (5), 399-402.
164. Clark, C. G., Extensive genetic diversity in *Blastocystis hominis*. *Molecular and biochemical parasitology* **1997**, 87 (1), 79-83.
165. Bohm-Gloning, B., et al., Five subgroups of *Blastocystis hominis* from symptomatic and asymptomatic patients revealed by restriction site analysis of PCR-amplified 16S-like rDNA. *Trop Med Internat Health* **1997**, 2, 771-8.
166. Horiki, N., et al., Epidemiologic survey of *Blastocystis hominis* infection in Japan. *Am J Trop Med Hyg* **1997**, 56 (4), 370-4.
167. Jelinek, T., et al., The role of *Blastocystis hominis* as a possible intestinal pathogen in travellers. *The Journal of infection* **1997**, 35 (1), 63-6.
168. Cimerman, S., et al., Blastocystosis: nitazoxanide as a new therapeutic option. *Revista da Sociedade Brasileira de Medicina Tropical* **2003**, 36 (3), 415-7.
169. Levy, Y., et al., Severe *Blastocystis hominis* in an elderly man. *The Journal of infection* **1996**, 33 (1), 57-9.
170. Jones, M. S., 2nd, et al., Detection of *Blastocystis* from stool samples using real-time PCR. *Parasitol Res* **2008**, 103 (3), 551-7.
171. Sekar, U.; Shanthi, M., *Blastocystis*: Consensus of treatment and controversies. *Trop Parasitol* **2013**, 3 (1), 35-9.
172. Ertug, S., et al., The effect of trimethoprim-sulfamethoxazole in *Blastocystis hominis* infection. *Turkiye Parazit Derg* **2009**, 33 (4), 270-2.
173. Silberman, J. D., et al., *Dientamoeba fragilis* shares a recent common evolutionary history with the trichomonads. *Molecular and biochemical parasitology* **1996**, 76 (1-2), 311-4.
174. Johnson, E. H., et al., Emerging from obscurity: biological, clinical, and diagnostic aspects of *Dientamoeba fragilis*. *Clin Microbiol Rev Jul* **2004**, 17, 553-70.
175. Stark, D., et al., Description of *Dientamoeba fragilis* cyst and precystic forms from human samples. *J Clin Microbiol* **2014**, 52 (7), 2680-3.
176. Stark, D., et al., A review of the clinical presentation of dientamoebiasis. *Am J Trop Med Hyg* **2010**, 82 (4), 614-9.
177. Cuffari, C., et al., *Dientamoeba fragilis* masquerading as allergic colitis. *J Pediatr Gastroenterol Nutr* **1998**, 26 (1), 16-20.
178. Peek, R., et al., Direct amplification and genotyping of *Dientamoeba fragilis* from human stool specimens. *Journal of clinical microbiology* **2004**, 42 (2), 631-5.
179. Roser, D., et al., Metronidazole therapy for treating dientamoebiasis in children is not associated

- with better clinical outcomes: a randomized, double-blinded and placebo-controlled clinical trial. *Clin Infect Dis* **2014**, 58 (12), 1692-9.
180. van Hellemond, J. J., et al., Is paromomycin the drug of choice for eradication of Blastocystis in adults? *J Infect Chemother* **2013**, 19 (3), 545-8.
181. Nagata, N., et al., *In vitro* susceptibility testing of *Dientamoeba fragilis*. *Antimicrob Agents Chemother* **2012**, 56 (1), 487-94.



Eloise Blaine Cram, Ph.D. (1896-1957)

Dr. Cram is remembered as a prominent American zoologist and parasitologist. She began her career at the USDA but then went on to join the Zoology Lab at the National Institutes of Health where she worked for twenty years. Dr. Cram was initially tasked with the problem of parasites in poultry and became world-renowned for her work in this area. Dr. Cram made her major contributions in the area of schistosomiasis where she focused on the critical role of snails in the life cycle of these deadly parasites. She was prolific, with over 160 papers to her name, and became an authority on parasitic diseases. In 1955 she served as the president of the American Society of Parasitologists.

15. Non-Pathogenic Protozoa

Introduction

We are constantly confronted with many microbes whose sole purpose seems to be to colonize us or to parasitize and take advantage of our biochemical systems. The human body can be viewed as a series of ecological niches that select numerous entities, including viruses, bacteria, fungi, protozoa, helminths, and arthropods. They enter through the gastrointestinal (GI), urogenital, and respiratory tracts through abrasions and other portals of entry. Most of the world's microbes are repelled and cannot remain on or within these environments. This is mainly due to the inadequacy of their fundamental biological makeup, preventing them from thriving on or in us, the resiliency of our existing microbiome, and the intricate human immune system.¹

Most of those who take up residence do us little or no harm. In fact, by some estimates, most cells on and in us are non-human! We refer to them collectively as our microbiome. Commensals do us no harm and are just along for the ride. Symbionts actively help maintain our homeostatic mechanisms. For example, the oral cavity harbors hundreds of different species of bacteria, excluding those leading to various states of ill health. Our intestinal tract is another good example of peaceful co-existence between our symbiotic microbes and us, harboring hundreds of species of “friendly” bacteria.²

A few that have managed to run the gauntlet of our immune system and overcome the physiological barriers established by our complex metabolic regimes can, and often do, cause pathology leading to clinical conditions. This chapter briefly mentions a few of those eukaryotic organisms that we routinely harbor, and which do us no harm. At some

point, the clinician may receive a laboratory result with the name of one or more of these commensals. The subject of this brief chapter is how these “hitchhiker” species should be approached in the context of the clinical setting.

Several commensal protozoans have been selected for life within us. Under unusual conditions, a few are associated with disease. When a person is placed at risk from infection (e.g., surgery, immunosuppression, or infection with another pathogenic organism), some commensal organisms become opportunistic pathogens, growing and extending their territory at the expense of our now compromised microbiome. At those times, the clinician has difficulty determining who did what to whom. The diagnostic microbiology laboratory now assumes a major role in helping to catalog microbes into the good, the bad, and the ugly. Resolving the disease's primary cause often reverses the opportunist's growth pattern. These organisms, including *Entamoeba dispar* and *E. gingivalis*, are rarely associated with actual infection.^{3,5}

The clinician must recognize that even though the organism reported is not a pathogen, it is potentially a marker of the patient's exposure to a situation that may have led to the acquisition or imbalance of another organism that may be pathogenic. The search should focus on all other agents transmitted by the same route. A representative of each organism mentioned in the following summaries can be found in Appendix C.

Commensal flagellated protozoa

Trichomonas tenax (\trick-oh-MOAN-us\, \tri-kə-'mō-nəs\), *T. hominis*, *Enteromonas hominis* (\EN-ter-owe-MOW-nas\, \ent-ə-'rō-'mō-'nas\), *Retortamonas intestinalis*, and *Chilomastix mesnili* all only colonize the human host, and are considered non-pathogenic by all standard criteria.⁶ *T. tenax*

lives in the oral cavity in plaque; the rest are intestinal dwellers. Only *C. mesnili* has a recognized cyst stage. All are considered amitochondriate, aerotolerant anaerobic protists.⁷

Pathogenic outcomes have been described where heavy growth of *T. tenax* was found concurrently with abscesses and tumors of the oral cavity.^{8,9} In addition, *T. tenax* has been isolated from cases of inhalation pneumonia and pleural effusions from a patient in which ulceration of the esophagus resulted in communication with the pleural cavity. A PCR test for detecting *T. tenax* in dental plaque has been reported.^{10,11} Due to the overwhelming number of people harboring this flagellate who do not experience any discomfort, *T. tenax* remains on the list of commensals.

A case of *E. hominis* has been reported in which the patient experienced diarrhea and was treated successfully with metronidazole.¹² Neither *R. intestinalis* nor *C. mesnili* have been linked to abnormal health conditions.

Commensal amoebae

Entamoeba dispar (\ent-a-MEE-ba\, \en-tə-'mē-bə\), *Entamoeba hartmanni*, *Entamoeba coli*, *Endolimax nana* (\en-DOW-lie-MAKS\, \en-də-'lī-'maks\), and *Iodamoeba bütschlii* are organisms often identified in routine stool examination and in general are not the cause of disease. A fecal examina-

tion reporting these organisms usually elicits confusion among clinicians seeking their patients' causes of diarrheal disease. In some cases, this can trigger unnecessary treatment or referral to a specialist who can clarify their significance. Some (e.g., *E. dispar* or *E. hartmanni*) resemble *Entamoeba histolytica*, especially to the inexperienced laboratory technician, who sometimes errs on the side of this pathogen rather than the commensal. Hence, the patient receives treatment for an entity that is not causing the problem. The illness often recurs after "treatment," and drug failure is blamed, frequently leading to rounds of unnecessary treatment. These commensal amoebae usually do not respond to the standard drugs used to eradicate *E. histolytica*. The use of PCR is recommended for the differentiation of these from pathogenic amoebae.¹³ Another approach uses monoclonal antibodies to distinguish *E. histolytica* cysts from those of *E. dispar* and other commensal amoebae, facilitating their use in an antigen-capture mode for routine diagnosis.¹⁴

Entamoeba polecki is an inhabitant of the gut tract of pigs that sometimes finds its way into humans. *Entamoeba gingivalis* lives in the gingival flaps of a small subset of humans that is not yet defined and is associated with but does not cause pyorrhea (periodontitis). Following radiation therapy, *E. gingivalis* was diagnosed by fine needle aspiration of a neck abscess.¹⁵

References

1. Bull, M. J.; Plummer, N. T., Part 1: The Human Gut Microbiome in Health and Disease. *Integr Med (Encinitas)* **2014**, *13* (6), 17-22.
2. Qin, J., et al., A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **2010**, *464* (7285), 59-65.
3. Allison-Jones, E., et al., *Entamoeba histolytica* as a commensal intestinal parasite in homosexual men. **1986**, *315*, 353-6.
4. Haque, R., et al., Short report: detection of *Entamoeba histolytica* and *E. dispar* directly in stool. *Am J Trop Med Hyg* **1994**, *50* (5), 595-6.

5. Lucht, E., et al., *Entamoeba gingivalis* in human immunodeficiency virus type 1-infected patients with periodontal disease. *Clin Inf Dis* **1998**, 27 (3), 471-3.
6. Aucott, J. N.; Ravdin, J. I., Amebiasis and “nonpathogenic” intestinal protozoa. *Infectious disease clinics of North America* **1993**, 7 (3), 467-85.
7. Coombs, G. H., et al., Evolutionary Relationships Among Protozoa 1998; p 110-132.
8. Duboucher, C., et al., Salivary trichomoniasis. A case report of infestation of a submaxillary gland by *Trichomonas tenax*. *Archives of pathology & laboratory medicine* **1995**, 119 (3), 277-9.
9. Shiota, T., et al., *Trichomonas tenax* empyema in an immunocompromised patient with advanced cancer. *Parasite (Paris, France)* **1998**, 5 (4), 375-7.
10. El Kamel, A., et al., Pulmonary eosinophilia due to *Trichomonas tenax*. *Thorax* **1996**, 51 (5), 554-5.
11. Kikuta, N., et al., Specific and sensitive detection of *Trichomonas tenax* by the polymerase chain reaction. *Lett Appl Microbiol* **1999**, 24 (3), 193-7.
12. Spriegel, J. R., et al., Infectious diarrhea secondary to *Enteromonas hominis*. *The American journal of gastroenterology* **1989**, 84 (10), 1313-4.
13. Acuna-Soto, R., et al., Application of the polymerase chain reaction to the epidemiology of pathogenic and nonpathogenic *Entamoeba histolytica*. *Am J Trop Med Hyg* **1993**, 48 (1), 58-70.
14. Walderich, B., et al., Development of monoclonal antibodies specifically recognizing the cyst stage of *Entamoeba histolytica*. *Am J Trop Med Hyg* **1998**, 59 (3), 347-51.
15. Perez-Jaffe, L., et al., *Entamoeba gingivalis* identified in a left upper neck nodule by fine-needle aspiration: a case report. *Diagnostic cytopathology* **1998**, 18 (6), 458-61.



Charles Donovan, M.D. (1863–1951)

Donovan identified the amastigotes of an infectious agent in spleen and white blood cells obtained from a young boy suffering from Kala-azar (a condition widely known but of unknown etiology at the time) while working in the British Medical Service in Madras, India. He wrote up his results and submitted them to the British Medical Journal. Three years earlier, William Boog Leishman had made similar observations from a British soldier in Dum Dum, West Bengal, India, and wrote a description nearly identical to the one generated by Donovan. Leishman also submitted his findings to The British Medical Journal back in England. Ronald Ross, then editor of that publication, deduced that each physician had discovered the exact same entity. Slides sent to him by Donovan confirmed the diagnosis as a new parasitic infection. Ross named it *Leishmania donovani* in honor of both physicians.

V. The Nematodes

Nematodes are non-segmented roundworms belonging to the phylum Nematoda and are Earth's most abundant multicellular organism. Most nematode species, 27,000+ described so far, are parasitic, infecting various plants and animals. The remainder of the species is free-living, occupying essential niches in soil, freshwater, saltwater, and other, more specialized habitats. For example, nematode species have been identified in benthic samples from the Mariana Trench (36,200 feet deep). Nematodes play major roles in ecological processes. For example, soil nematodes are essential for helping to maintain soil fertility by ingesting soil microbes and excreting solid wastes that serve directly as plant nutrients. Nematodes are so abundant, given their ecologic flexibility, that if all the inert matter of soil could be removed from the Earth, it would still look about the same as seen from the International Space Station.¹

Most parasitic nematodes have evolved to infect a single host species and are incapable of survival in any other. Several have succeeded in adapting to multiple host species. Nearly every group of arthropods and vertebrates is infected with host-specific species of nematodes. Fortunately, only a small fraction of Nematode species is parasitic in humans. Yet, the human diseases caused by nematodes are often neglected and cause a high degree of morbidity, such as blindness and the disfiguring disease colloquially referred to as elephantiasis and chronic anemia from hookworm infection. The WHO NTD roadmap was developed and released in 2020 as a guide to elimination of the diseases caused by nematode organisms that remain neglected and each will be discussed in the relevant chapters to follow.²

More is known about the biology of *Caenorhabditis elegans*, a soil-dwelling spe-

cies more easily cultured under laboratory conditions than any other nematode. Its entire genome has been sequenced, encoding 20,512 genes. In contrast, the genome of *Trichinella spiralis*, a widespread parasitic nematode, although it has more total DNA than *C. elegans*, only 60% of it is homologous with its free-living relatives. *T. spiralis* has 16,380 coding regions, implying that this parasite requires fewer, not more, genes than free-living roundworms. The relationship of genes and the link to parasitism for nematodes is facilitated by genomic sequencing, and in the case of *T. spiralis*, virulence factors and other specialized compounds needed to resist digestion or immune attack are likely to be encoded by genes that permit *Trichinella* to live comfortably for years within its mammalian host muscle tissue in the face of an exquisitely developed host immune system.³

Nematode infections in humans are prevalent worldwide, with some species affecting nearly all of us at some time during our lifetime. Globally, the most diagnosed nematode infections are three types of soil-transmitted helminths (STHs), the giant intestinal roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*), and the hookworms (*Necator americanus* and *Ancylostoma duodenale*). Children are particularly susceptible to acquiring large numbers of these parasites and consequently suffer greater morbidity than adults harboring the same parasites. In many developing countries, children frequently harbor all three types of STHs (hence the moniker “the unholy trinity”) and, as a result, often exhibit evidence of malnutrition, physical growth retardation, and cognitive and intellectual development deficits.

The typical nematode, both larva and adult, is surrounded by a flexible, durable outer coating, the acellular cuticle, resistant to various chemicals commonly found in their everyday environment. The cuticle is a complex

structure composed of various layers with many components, including structural proteins, enzymes, and lipids. The impermeable cuticle of each species is mainly composed of several unique types of collagen. A further layer, the epicuticle, surrounds the cuticle of a few parasitic species, making them even more resistant to attack from host resistance factors, including digestive enzymes and antibodies.

All nematodes have a well-developed nervous and muscular system. The longitudinal striated skeletal muscular system of nematodes lies just underneath the cuticle; their origins and insertions are in cuticular processes. Some muscle tissue surrounds the buccal cavity and esophageal and sub-esophageal regions of the gut tract. These muscles are particularly important feeding apparatus elements in parasitic and free-living nematodes. Each muscle cell consists of filaments (actin and myosin), mitochondria, and cytoplasmic processes that connect it with a single nerve fiber.

The nervous system consists of a dorsal nerve ring or a series of ganglia that give rise to the peripheral nerves - two lateral, one dorsal, and one ventral branch. Commissures connect the branches and allow for signaling integration, resulting in fluid, serpiginous movements. Many nematode species have specialized nerve endings, termed amphids and phasmids, that terminate at the cuticle's exterior at the organism's head and tail, respectively. This allows them to sense their external environment; some play a central role in mating behavior. Pheromones produced by parasitic nematodes of both sexes are species-specific chemical attractants, facilitating the worm's efforts to find a mate within the host.⁴ Several classes of drugs interfere specifically with nematode nervous system signaling molecules and effectively treat infections in domestic cattle and humans.

Nematodes have a complete, functional gut tract: the oral (i.e., buccal) cavity and esophagus, the midgut, and the hindgut with anus. The cuticle usually lines the oral cavity and hindgut; the midgut consists of columnar cells, complete with microvilli. The function of the midgut is to absorb ingested nutrients, whereas the usually muscular esophagus serves to deliver food to the midgut. In addition, several specialized exocrine glands open into the lumen of the digestive tract, usually in the region of the esophagus. These glands are largely concerned with digestion but may also have other functions. For example, the cephalic glands of hookworm adults secrete a highly effective anticoagulant, allowing it to feed continuously on blood from the host. Hookworms then digest hemoglobin through a series of enzymatic breakdown pathways in the parasite gut, followed by heme detoxification. These pathways are now being exploited to shape new vaccination strategies.⁵

In other instances, a single row of cells called *stichocytes* empty their products directly into the esophagus via a cuticular-lined duct. These cells occupy a large portion of the body mass of *Trichinella*, *Trichuris*, and *Capillaria*. These cells' function(s) are not fully understood and may vary from species to species.

Nematodes excrete solid and fluid wastes. Excretion of solids takes place through the digestive tract. Fluids are eliminated using the excretory system, consisting of two or more collecting tubules connected at one end to the ventral gland (a primitive kidney-like organ) and at the other end to the excretory pore that leads to the external environment.

The adult stage typically consists of both males and females. Most species of nematode require that the male transfers amoeboid-like sperm to the female during mating, followed by the fusion of the sperm with the ovum to

begin the process of embryogenesis. A large portion of the female nematode's anatomy is devoted to reproduction. One or two ovaries lead to the vagina through a tubular oviduct and uterus. A seminal receptacle for the storage of sperm is connected to the uterus. The male has a single testis connected to the vas deferens, seminal vesicle, ejaculatory duct, and cloaca. In addition, males of many species have specialized structures (e.g., claspers, spicules, or a hand-like bursa) to aid in transferring sperm to the female during mating. Their taxonomic identification is often based on the morphology of these structures. Most nematodes lay eggs, but some are viviparous.

All nematodes exhibit a punctuated form of life cycle, beginning with the egg or larval stage. The immature L1 stage undergoes a series of molts in which its cuticle is shed,

and a new larger cuticle is synthesized to sheath the next stage. A total of four molts (L1 to L2 to L3 to L4) culminates in the mature adult worm. In most nematode species, some growth and differentiation usually occur between each molt. If embryonated eggs are the infectious stage, then transmission. If an embryonated egg is the infectious stage, then transmission is by the oral route. Several groups of nematodes depend upon insect vectors for their transmission. In these instances, it is the L3 larva that is the infectious stage for the definitive host. A few are transmitted from host to host by scavenging an infected, dead host.

The text discusses further details regarding the biology of each nematode parasite whenever it relates to the pathogenesis of the disease.

References

1. Schratzberger, M., et al., A Worm's World: Ecological Flexibility Pays Off for Free-Living Nematodes in Sediments and Soils. *Bioscience* **2019**, 69 (11), 867-876.
2. Organization, W. H., Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva. Licence: CC BY-NC-SA 3.0 IGO **2020**.
3. Viney, M., The genomic basis of nematode parasitism. *Brief Funct Genomics* **2018**, 17 (1), 8-14.
4. Yang, B., et al., Nematode Pheromones: Structures and Functions. *Molecules* **2023**, 28 (5).
5. Puchner, K. P., et al., Vaccine value profile for Hookworm. *Vaccine* **2023**.

16. *Enterobius vermicularis*

(Linnaeus 1758)

Pronunciation: \ent-ə-'rō-bē-əs\

Introduction

Enterobius vermicularis (\EN-ter-owe-BEE-us\), commonly known as pinworm, is the most prevalent nematode infection of humans, its only host. In the United States, infection with pinworm may affect up to 40 million individuals, or more than 10% of the population.¹ It is likely that the prevalence of enterobiasis has diminished considerably over the last decade. In some European communities, the prevalence rates may be as high as 50% in children, especially in the poorer countries of Eastern Europe and the Balkans.^{1,2} *E. vermicularis* is mainly an infection of school-aged children, but infections have been diagnosed in the elderly and certain other populations, such as institutionalized and immunosuppressed individuals.^{3,6} Transmission of pinworm is especially frequent in elementary schools and daycare centers.⁶ A syndrome of eosinophilic colitis associated with *E. vermicularis* larvae has been described but is notable for not causing peripheral eosinophilia.⁷

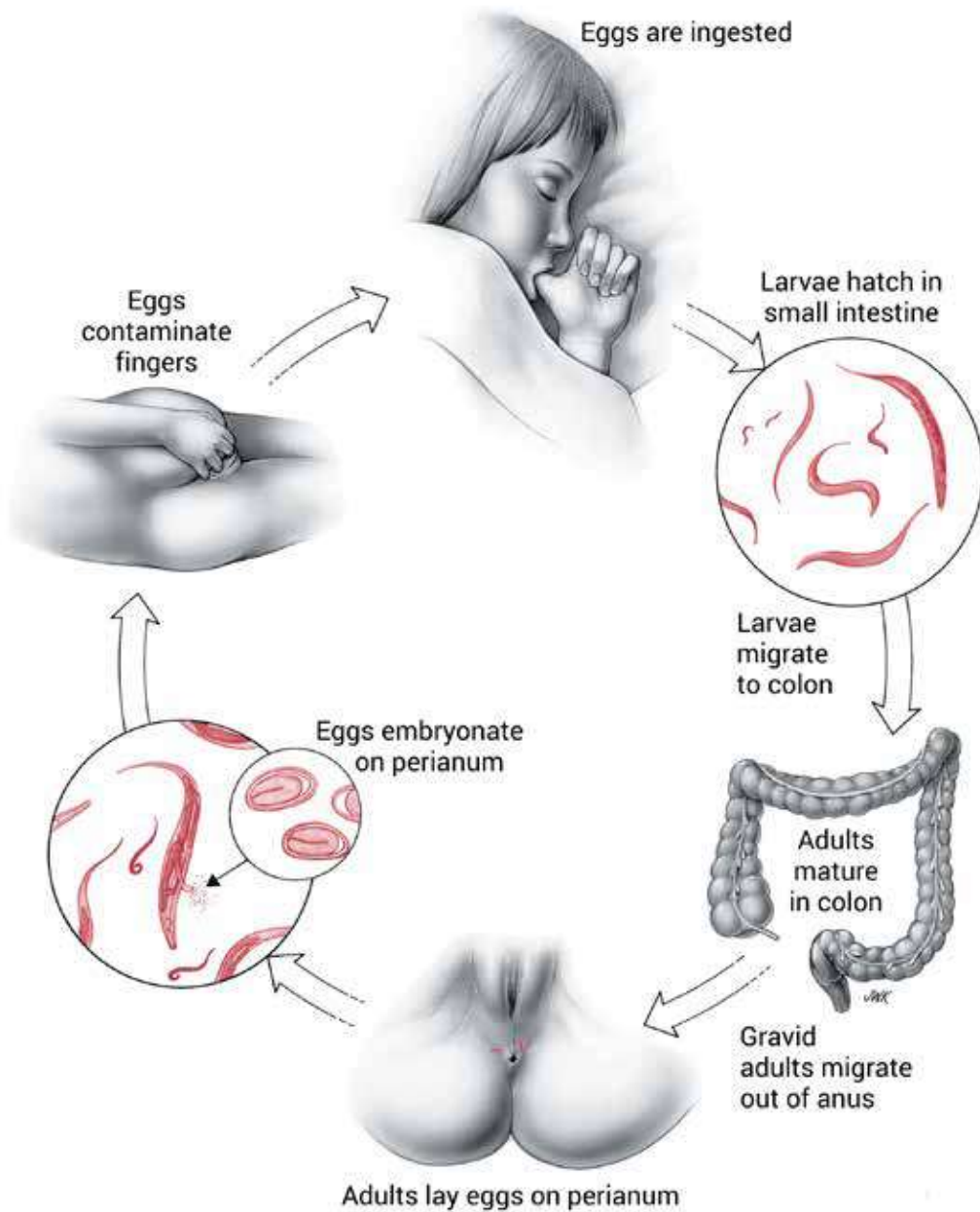
Historical Information

In 1758, Carl Linnaeus named this organism *Enterobius vermicularis*.⁸ In 1824, Johann Bremser distinguished this roundworm from the other oxyurid and ascarid nematodes and accurately described it, forming the basis for today's modern classification scheme.⁹ Pinworm ova have been recovered from human coprolites found in numerous archeological sites, some as old as 10,000 years. *Enterobius* DNA has been detected in ancient DNA from North and South American human coprolites.^{10, 11}



Figure 16.1. Adult female *Enterobius vermicularis*. 10mm.

Enterobius vermicularis



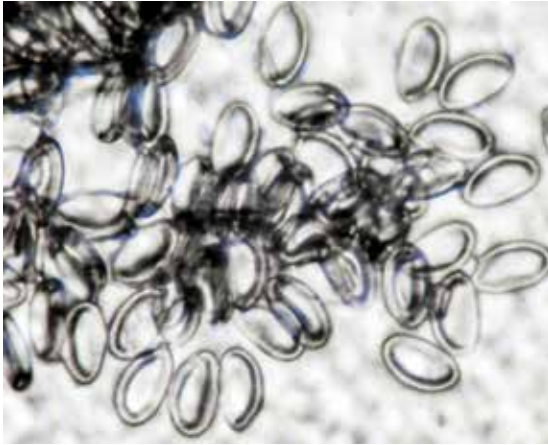


Figure 16.2. Embryonated eggs of *E. vermicularis*.

Life Cycle

The pinworm lifecycle is one of the simplest among all nematode parasites, reflecting a typical pattern of development: four larval stages (L1–L4) and the adult stage. Adults live freely in the lumen of the transverse and descending colon and in the rectum. The female (Fig. 16.1) measures 8–13 mm by 0.3–0.6 mm. The female's tail comes to a sharp point, hence the common name pinworm. The male is typically smaller, measuring 2–5 mm by 0.2 mm. The tail of the male contains a single curved copulatory spicule. Adult pinworms feed on our microbiome.

Adult worms mate and, six weeks later, produce approximately 10,000 fertilized, non-embryonated eggs within each female. Males die shortly after copulation. The gravid female migrates out of the anus onto the perianal skin at night, most likely after receiving an environmental cue from the host, a drop in body temperature. There, she experiences a prolapse of her uterus, expels all her eggs, and then dies. Expulsion can be so intense that the eggs become airborne. The fertilized eggs rapidly develop and are infectious within 6 hours after being laid, exhibiting one of the fastest embryological developmental cycles among all nematodes.

An uncomfortable perianal pruritus develops, called *pruritus ani*, that may be severe enough to cause sleep disturbances.¹² Scratching of the perianal area can often lead to eggs lodging under the fingernails. Ingestion of eggs can occur when a child places contaminated hands into their mouth. The embryonated eggs (Fig. 16.2) are swallowed and hatch as L1 larvae. Once they reach the small intestine, they shed their cuticle (molt), becoming L2 larvae. Development to the third and fourth stages also occurs in the small intestine. L4 larvae feed on bacteria and then molt, transforming into adults that travel to the large intestine and appendix (Figure 16.3), where they reside. The entire cycle is completed within 4–6 weeks after ingesting the infectious egg. Alternatively, eggs can hatch on the skin at the deposition site, and the L2 larvae can crawl back through the anus into the rectum and eventually the colon, where they develop into reproducing adults. This is referred to as retro-infection (a form of autoinfection).

In female patients, the larvae that hatch on the skin near the anus occasionally crawl into the vagina instead of the rectum, establishing an aberrant infection. Less frequently, gravid adult worms infect the fallopian tubes. Aberrant infections can result in pelvic peritonitis, ovarian infection, and liver granuloma.^{13–18}

Cellular and Molecular Pathogenesis

All stages of *E. vermicularis* develop in the lumen of the gastrointestinal (GI) tract, so the host does not experience any systemic reactions unless the worm burden is particularly high or there is an ectopic infection. The parasite elicits a mild, local inflammatory response, and while eosinophilic colitis has been described, circulating eosinophilia does not develop.^{7,19}

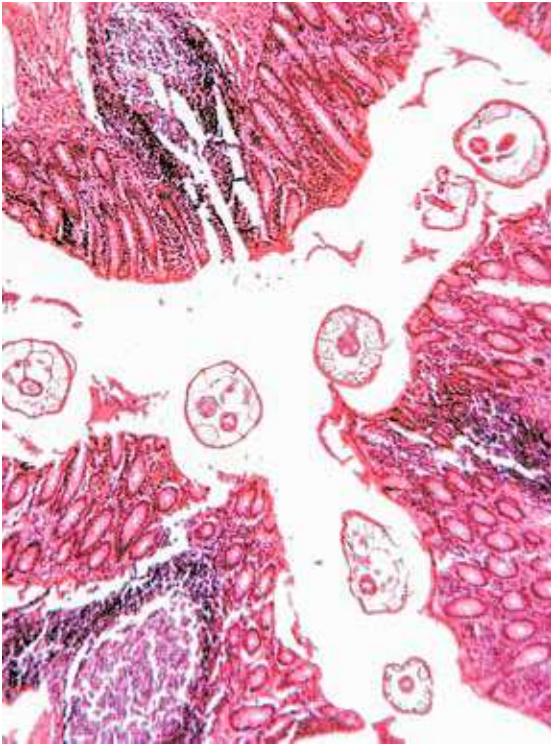


Figure 16.3. Cross sections of adult *E. vermicularis* in appendix.

A few patients develop pruritus resulting from allergic responses to worm proteins. Whether pinworm infection causes secondary problems, such as appendicitis or pelvic inflammatory disease, remains unresolved.¹⁷ Pinworms have been found in these organs at autopsy with no evidence of an inflammatory reaction. In other circumstances, pinworms have been implicated in the chain of events leading to clinical appendicitis.²⁰

Although there are no comparable studies in humans, experimental evidence has shown that the host's immune status affects the outcome of the infection. *Syphacia oblevata* is a pinworm species that only infects mice and reaches much larger numbers in nude (athymic) mice than in the same mice into which a subcutaneous implant of thymic tissue from syngeneic donors was transplanted before infection.²¹ In one unusual case, intense infiltration of the colon with eosinophils and neutrophils led to clinical eosinophilic enteritis

in an 18-year-old male who passed numerous *E. vermicularis* larvae. The larvae were definitively identified based on characteristic 28S rRNA and 5S rRNA spacer genes by PCR.⁹ Susceptibility to pinworm infection decreases with age in humans, but the reasons for this are not clear. Whether this difference in susceptibility has an immunological or physiological basis remains to be determined.

Clinical Disease

All stages of *E. vermicularis* develop in the lumen of the gastrointestinal (GI) tract, so the host does not experience any systemic reactions unless the worm burden is particularly high or there is an ectopic infection. The parasite elicits a mild, local inflammatory response, and while eosinophilic colitis has been described, circulating eosinophilia does not develop.^{7, 19}

A few patients develop pruritus resulting from allergic responses to worm proteins. Whether pinworm infection causes secondary problems, such as appendicitis or pelvic inflammatory disease, remains unresolved.¹⁷ Pinworms have been found in these organs at autopsy with no evidence of an inflammatory reaction. In other circumstances, pinworms have been implicated in the chain of events leading to clinical appendicitis.²⁰

Although there are no comparable studies in humans, experimental evidence has shown that the host's immune status affects the outcome of the infection. *Syphacia oblevata* is a pinworm species that only infects mice and reaches much larger numbers in nude (athymic) mice than in the same mice into which a subcutaneous implant of thymic tissue from syngeneic donors was transplanted before infection.²¹ In one unusual case, intense infiltration of the colon with eosinophils and neutrophils led to clinical eosinophilic enteritis

in an 18-year-old male who passed numerous *E. vermicularis* larvae. The larvae were definitively identified based on characteristic 28S rRNA and 5S rRNA spacer genes by PCR.⁹ Susceptibility to pinworm infection decreases with age in humans, but the reasons for this are not clear. Whether this difference in susceptibility has an immunological or physiological basis remains to be determined.

Diagnosis (see Clinical Appendix)

Infection is usually diagnosed by visualization of pinworm eggs or adult worms. Since eggs are deposited on the perianal skin and not released into feces, stool examination for ova and parasites is useless in diagnosing this infection. Eggs are best obtained from the perianal area using clear (not frosted) adhesive tape or the commercially available adhesive pinworm paddle. Parents of children to be examined are typically instructed by a physician in using either diagnostic aid. The adhesive tape or paddle should be applied to the perianal region in the early morning hours as the person sleeps or as soon as they awake (i.e., before a bath or bowel movement). The tape or paddle is then examined using light microscopy. The characteristic eggs (Figs. 16.2, C.37) can be readily detected in this manner. Occasionally, thread-like female worms may be directly visible on the perianal skin. Female worms are 8–13mm long and thin, appearing as small white pieces of thread. Serologic tests specific for *E. vermicularis* are not used clinically to diagnose this infection.

Adult pinworms can be readily identified when they are seen on histologic sections because of bilateral cuticular projections known as alae. A fecal examination may be necessary in patients with abdominal pain or other GI symptoms to rule out co-infection with other infectious agents. A colonoscopy

of a patient with eosinophilic enteritis from *E. vermicularis* showed a purulent discharge from the rectum to the terminal ileum and ulcerations. One patient described with this syndrome was noted to pass larvae instead of eggs or adult worms, which required PCR for identification.⁷

Treatment (see Clinical Appendix)

Pyrantel pamoate in a single dose of 11 mg/kg (max. 1 gram), or either albendazole 400 mg or mebendazole 100 mg in a single dose is the recommended therapy, with improved efficacy approaching 100% if a second dose is given 2–3 weeks after the first.^{27–30} In the United States, pyrantel pamoate is an inexpensive and effective over-the-counter option. Whereas treatment with alternative medications, such as albendazole, prescribed by a physician unaware of drug prices, can cost hundreds of dollars. None of these drugs kills the eggs or developing larvae; therefore, “blind” re-treatment is the reason for a second treatment 2–3 weeks after the original therapy. This second round of therapy destroys worms that have hatched from eggs ingested after the first treatment.^{27–30} Since eggs can survive 2–3 weeks on clothing, inanimate objects, and bedding, reinfection can continue to occur only for this limited period, and one retreatment is usually sufficient. Knowing that the entire cycle takes 4–6 weeks after ingestion of the infectious egg can help identify any contacts that might be infected and identify the patient’s exposure. Since eggs can survive 2–3 weeks before being ingested, the timing of exposure for an infected patient is 1–2 months before the appearance of adult female worms capable of producing infective eggs. Treatment of exposed contacts, all household members, and source patients, if not household members, is recommended and has been successful in both households and institutions.

Prevention and Control

In a young child, a cycle of infection and reinfection is frequent because of the ready transmissibility of the pinworm. The groups showing the highest prevalence of infection are school children and institutionalized individuals. Compounding the problem is that the eggs can survive for several days under high humidity and intermediate to low temperatures. There are no predilections for higher rates of infections based on sex, race, or socioeconomic class.

Thorough washing of hands with soap and water after using the toilet and changing diapers by children or their caregivers should help to reduce transmission.³ Trimming of fingernails has been suggested to decrease the possibility of eggs collecting and to reduce the risk of skin breaks in the perianal area from scratching. Mass treatment during outbreaks can be successful in institutions, daycare centers, schools, or other areas with pinworm infections.³¹

References

- Burkhart, C. N.; Burkhart, C. G., Assessment of frequency, transmission, and genitourinary complications of enterobiasis (pinworms). *Int J Dermatol* **2005**, *44* (10), 837-40.
- Hotez, P. J.; Gurwith, M., Europe's neglected infections of poverty. *Int J Infect Dis* **2011**, *15* (9), e611-9.
- Snow, M., Pinning down pinworms. *Nursing* **2006**, *36* (5), 17.
- Agholi, M., et al., HIV/AIDS-associated opportunistic protozoal diarrhea. *AIDS Res Hum Retroviruses* **2013**, *29* (1), 35-41.
- Schupf, N., et al., Prevalence of intestinal parasite infections among individuals with mental retardation in New York State. *Ment Retard* **1995**, *33* (2), 84-9.
- Crawford, F. G.; Vermund, S. H., Parasitic infections in day care centers. *The Pediatric infectious disease journal* **1987**, *6* (8), 744-9.
- Liu, L. X., et al., Eosinophilic colitis associated with larvae of the pinworm *Enterobius vermicularis*. *Lancet (London, England)* **1995**, *346* (8972), 410-2.
- Cox, F. E., History of human parasitology. *Clin Microbiol Rev* **2002**, *15* (4), 595-612.
- Bremser, J. G., Oxyure vermiculaire. In *Traite zoologique et physiologique sur les vers intestinaux de l'homme CLF Panchouke Paris pp* **1824**, 149-157.
- Horne, P. D., First evidence of enterobiasis in ancient Egypt. *J Parasitol* **2002**, *88* (5), 1019-21.
- Iniguez, A. M., et al., *Enterobius vermicularis*: ancient DNA from North and South American human coprolites. *Mem Inst Oswaldo Cruz* **2003**, *98* Suppl **2003**, *1*, 67-9.
- Jones, J. E., Pinworms. *Am Fam Physician* **1988**, *38* (3), 159-64.
- Pearson, R. D.; Irons, R. P., Chronic pelvic peritonitis due to the pinworm *Enterobius vermicularis*. *JAMA* **1981**, *245* (13), 1340-1.
- Beckman, E. N.; Holland, J. B., Ovarian enterobiasis--a proposed pathogenesis. *The American journal of tropical medicine and hygiene* **1981**, *30* (1), 74-6.
- Daly, J. J.; Baker, G. F., Pinworm granuloma of the liver. *The American journal of tropical medicine and hygiene* **1984**, *33* (1), 62-4.
- Arca, M. J., et al., Clinical manifestations of appendiceal pinworms in children: an institutional experience and a review of the literature. *Pediatric surgery international* **2004**, *20* (5), 372-5.
- Lala, S.; Upadhyay, V., *Enterobius vermicularis* and its role in paediatric appendicitis: protection or predisposition? *ANZ J Surg* **2016**.
- Powell, G., et al., *Enterobius vermicularis* infection of the ovary. *BMJ Case Rep* **2013**, *2013*.
- Cacopardo, B., et al., Eosinophilic ileocolitis by *Enterobius vermicularis*: a description of two rare cases. *Ital J Gastroenterol Hepatol* **1997**, *29* (1), 51-3.
- Ahmed, M. U., et al., The Frequency of *Enterobius Vermicularis* Infections in Patients Diagnosed

- With Acute Appendicitis in Pakistan. *Glob J Health Sci* **2015**, 7 (5), 196-201.
21. Jacobson, R. H.; Reed, N. D., The thymus dependency of resistance to pinworm infection in mice. *The Journal of parasitology* **1974**, 60 (6), 976-9.
22. Wendt, S., et al., The Diagnosis and Treatment of Pinworm Infection. *Dtsch Arztebl Int* **2019**, 116 (13), 213-219.
23. Dezsényi, B., et al., Gynecological and obstetrical aspects of Enterobius vermicularis infection. *Acta Microbiol Immunol Hung* **2018**, 65 (4), 459-465.
24. Ockert, G., [Epidemiology of *Dientamoeba fragilis* Jepps and Dobell 1918. 1. Spread of the species in child collectives]. *Journal of hygiene, epidemiology, microbiology, and immunology* **1972**, 16 (2), 213-21.
25. Hotez, P. J., The other intestinal protozoa: enteric infections caused by Blastocystis hominis, Entamoeba coli, and Dientamoeba fragilis. *Sem Pedi Infect Dis* **2000**.
26. Yildiz, I., et al., Investigation of Dientamoeba fragilis Frequency in Faecal Samples of Patients with Enterobius vermicularis Infection by Polymerase Chain Reaction. *Turkiye Parazitol Derg* **2021**, 45 (3), 195-200.
27. St. Georgiev, V., Opportunistic infections: treatment and developmental therapeutics of cryptosporidiosis and isosporiasis. *Drug Develop Res* **1993**, 28, 445-59.
28. Wang, B. R., et al., Comparative efficacy of thienpydin, pyrantel pamoate, mebendazole and albendazole in treating ascariasis and enterobiasis. *Chin Med J (Engl)* **1987**, 100 (11), 928-30.
29. Lormans, J. A., et al., Mebendazole (R 17635) in enterobiasis. A clinical trial in mental retardates. *Chemotherapy* **1975**, 21 (3-4), 255-60.
30. St Georgiev, V., Chemotherapy of enterobiasis (oxyuriasis). *Expert Opin Pharmacother* **2001**, 2 (2), 267-75.
31. Ashford, R. W., et al., Enterobius vermicularis infection in a children's ward. *J Hosp Infect* **1988**, 12 (3), 221-4.

17. *Trichuris trichiura*

(Linnaeus 1771)

Pronunciation: \trik-'yür-əs\\tri-kē- ər-ə\\

Introduction

Trichuris trichiura (\\TRICK-you-ris\\trick-ee-UR-ah\\), commonly known as whipworm because of its characteristic shape, is one of the three major soil-transmitted helminths (STHs) that cause serious morbidity in developing countries.^{1,4} *Trichuris* infection is frequently coincident with infections caused by the other STHs, *Ascaris lumbricoides* and the hookworms.⁵ The prevalence of trichuriasis exceeds 500 million worldwide, with the largest number of infections occurring in Asia, Sub-Saharan Africa, and the tropical regions of the Americas.⁶ It is likely that trichuriasis still occurs in the southeastern region of the U. S.^{1-3, 7}

T. trichiura has no reservoir hosts. Other species of *Trichuris* infect a wide range of mammals (e.g., *T. vulpis* in Canidae, *T. muris* in the mouse, *T. suis* in the pig). Worm burdens are usually higher in children than in adults, and disease is consequently more severe in that age group.⁷ School-aged children are particularly affected. Heavily infected children often go on to develop colitis and stunted growth, and those with chronic infections can even develop intellectual and cognitive deficits.^{7, 8}

Historical Information

In 1740, Giovanni Morgagni was the first to describe *T. trichiura* in the cecum and transverse colon.⁹ In 1761, a report by Johannes Roederer, depicted the external morphology of *T. trichiura*.¹⁰ Roederer's report was accompanied by scientific renderings that are still deemed highly accurate. Carl Linnaeus classified this parasite, then called “teretes,” as a nematode in 1771.¹¹ The discovery of petri-



Figure 17.1. Adult female *Trichuris trichiura*.

fied eggs in coprolites of prehistoric humans has confirmed it to be a human pathogen for over 5,000 years.¹¹

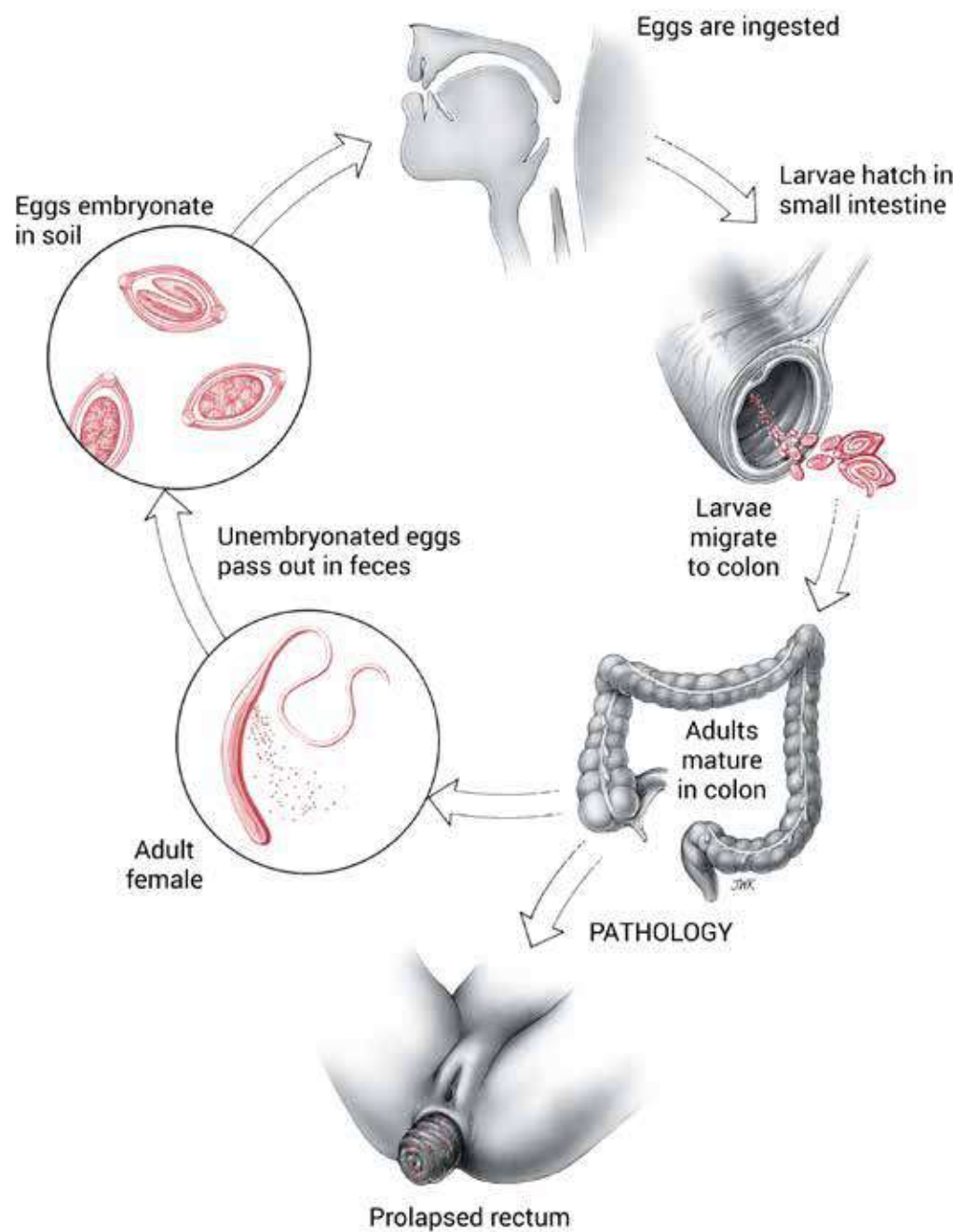
Life Cycle

The adult female (Fig. 17.1) measures 30–50 mm, while the male (Fig. 17.2) is 30–45 mm in length. Infection begins when the embryonated egg is swallowed. The exact process of site selection in the host, from ingestion of embryonated eggs to the presence of adult worms, is not entirely clear. One interpre-



Figure 17.2. Adult male *Trichuris trichiura*.

Trichuris trichiura



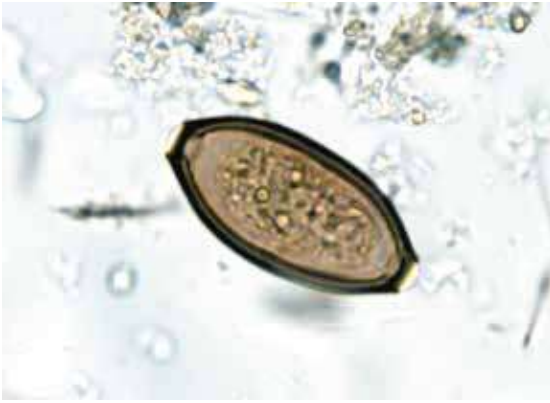


Figure 17.3. Fertilized, non-embryonated egg of *T. trichiura*. 50 μm x 20 μm .

tation of the data suggests that the L1 larva hatches in the small intestine penetrates the columnar epithelium and lies just above the lamina propria. Four molts later, the immature adult emerges and is passively carried to the large intestine, where it re-embeds in the columnar cells. Others challenge the importance or presence of the “duodenal phase” in human *T. trichiura* infection. This alternative theory proposes that sequential exposure to gastric, pancreatic, and ileal secretions, followed by exposure to the colonic environment, results in the larvae hatching that ultimately invade the columnar cells of the colon.¹²

Despite this controversy regarding the life cycle steps from ingestion to invasion of the columnar cells of the colon, adult *T. trichiura* live in the transverse and descending colon (Fig 17.4). The adult worm’s anterior, narrow, elongate esophagus is embedded within a syncytium of host cells induced by the worm. This syncytium probably results from exposure of the host to worm secretions emanating from its stichosome. The posterior abdomen protrudes into the lumen, allowing eggs to escape. Nothing is known about the nutritional requirements of this parasite, but experimental evidence on related species suggests that they do not ingest blood.¹³ The parasites grow and mature in the large intestine, where mating also occurs.

Patency (i.e., the first-time eggs are detectable in the feces) is about 90 days after ingesting embryonated eggs. Females can produce up to 3,000–5,000 eggs per day and live for 1.5–2.0 years.^{14, 15} Fertilized eggs are deposited in soil with feces and must embryonate there before becoming infectious. Environmental factors, including high humidity, sandy or loamy soil, and a warm temperature (20–30 °C), favor rapid development of the embryo.¹⁶ Under optimal conditions, embryonic development takes place over an 18–22 day period.¹⁷

Cellular and Molecular Pathogenesis

In *Trichuris*-endemic areas, pediatric populations typically harbor the largest numbers of worms, with the highest burdens in children between ages 5–15 years. It is unknown why these heavy worm burdens diminish in older age groups. Some studies indicate that susceptibility to heavy *Trichuris* infections may depend on an inability to mount a strong T helper cell type 2 response.¹⁸ There is also epidemiological evidence for a genetic component to susceptibility.¹⁹

Adult whipworms in the large intestine

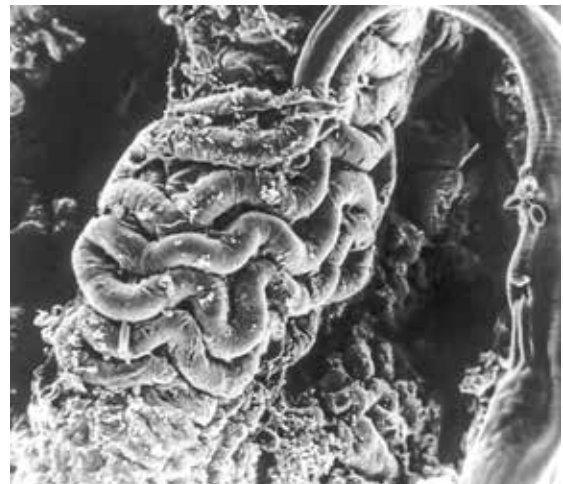


Figure 17.4. Scanning EM of adult *Trichuris*, *in situ*. Courtesy K. Wright.



Figure 17.5. Prolapsed rectum with adult *T. trichiura*.

induce structural defects in the epithelium.²⁰ To invade the colonic mucosa, adult *Trichuris* release novel pore-forming and channel-forming proteins.²¹ *In vitro*, they induce ion-conducting pores in lipid bilayers. Pore formation in epithelial cell membranes may facilitate invasion and enable the parasite to maintain its syncytial environment in the cecal epithelium. Genes encoding these novel proteins are comprised of repeats.²²

Despite the immunomodulatory capacity of adult *Trichuris*, in some cases, a low-grade inflammatory response to the presence of the adults can occur. This, together with the upregulation of inflammatory biomarkers, can present a clinical picture resembling inflammatory bowel disease, ulcerative colitis, or Crohn's disease.²³ The latter conditions are characterized by more extensive histopathologic damage to the gut. With heavy infections, the population of whipworms may extend from the proximal to the terminal end of the ileum and cause ileitis. Anemia results from a combination of capillary damage and erosion, leading to blood loss and anemia of chronic inflammation.²³ Generally, the anemia resulting from heavy infection with *T. trichiura* is much less severe than hookworm-related anemia.

In contrast to situations with low-grade inflammation, adult *Trichuris* immunomodulatory effects typically predominate. Ironically, these features of the whipworm have been exploited to develop a novel treatment for Crohn's disease. Ingestion of embryonated eggs of the porcine whipworm *T. suis* has been shown to reduce the symptoms of Crohn's disease for short periods without significant adverse effects on the patient.^{24, 25} The precise mechanism of how *T. suis* reduces host inflammation is being actively investigated, and current evidence suggests that *T. suis* shifts the immune system from a Th1 to a Th2 response and changes the levels of certain cytokines, possibly in part through changes in the gut microbiome.^{26, 27} Research suggests that excreted/secreted products affect intestinal epithelium, macrophage, and dendritic cells and suppress the pro-inflammatory cytokine production by these cells.²⁸⁻³⁰ Gastrointestinal parasites can also influence the apoptosis of intestinal cells creating a complex interplay between parasites and their host.^{30, 31}

Clinical Disease

Clinical disease occurs mainly in children.³² Patients with heavy *Trichuris* infections may present with dysentery or chronic colitis. *Trichuris*-induced dysentery results in weight loss, emaciation, and anemia. Because of the extensive mucosal swelling of the rectum, the urge to strain as if feces were present (tenesmus) can occur. Protracted tenesmus can lead to rectal prolapse (Fig. 17.5).²⁰

Chronic *Trichuris* colitis in pediatric patients can resemble characteristics of better-known forms of inflammatory bowel disease, such as Crohn's disease and ulcerative colitis. Children suffering from heavy trichuriasis develop chronic malnutrition, short stature, anemia, and finger clubbing.²⁰ Following specific chemotherapy, many of these condi-

tions abate, often resulting in rapid catch-up growth.

Increasing evidence suggests that in addition to the physical symptoms of trichuriasis, chronic infection can also produce long-term deficits in child cognitive and intellectual development.⁸ The mechanism by which this occurs is not yet known.

Diagnosis (see Clinical Appendix)

Trichuris eggs have a characteristic appearance and are easily identified (Fig.). In cases of light infection, the concentration of feces before microscopic examination may be required to increase the chances of finding eggs. Identifying Charcot-Leyden crystals in the stool should lead to further examination, even without identifying eggs on a first stool examination. A search for pathogenic protozoa, such as *Entamoeba histolytica* or *Giardia lamblia*, is indicated, given the high frequency of multiple infections. While identifying *Trichuris* eggs is relatively easy, finding *Giardia* or *Entamoeba* requires an experienced microscopist. Adult *Trichuris* can also be identified by direct visualization on colonoscopy.³³ When doubt exists, it is reasonable to treat the patient for *Trichuris* infection and request expert advice if the patient's symptoms do not abate. Failure to control diarrhea after trichuriasis treatment mandates a more thorough evaluation of other causes of diarrhea. Stool cultures should be carried out to determine the possible presence of enteric prokaryotic or viral pathogens. In histologic preparations, *Trichuris* adults can be readily identified by the characteristic variability of their diameter.

Treatment (see Clinical Appendix)

A benzimidazole - mebendazole or albendazole

albendazole - is the treatment of choice for trichuriasis.¹ The primary mechanism of these drugs is to inhibit microtubule polymerization by affinity binding to the unique beta-tubulin of invertebrates. Although most global anthelmintic de-worming programs rely on using a single dose of either drug, several doses are usually required to effect a cure.³⁴ Alternatively, *Trichuris* deworming can be improved by adding ivermectin in high prevalence areas.³⁵ In Africa, it is currently common to combine albendazole with ivermectin in programs that simultaneously target intestinal helminth infections, including trichuriasis, with other helminth disease elimination programs for lymphatic filariasis or onchocerciasis.³⁶

Both albendazole and mebendazole have an excellent safety profile in children. In the doses used to treat STH infections, neither drug causes significant systemic toxicity in routine use, although transient abdominal pain, diarrhea, nausea, and dizziness have been reported. Long-term use has been associated with bone marrow suppression, alopecia and hepatotoxicity. There is a single report that albendazole resulted in impaired growth in children with asymptomatic trichuriasis, although this observation has not been confirmed in other studies.³²

Mebendazole and albendazole are teratogenic and embryotoxic in pregnant laboratory rats at 10 mg/kg doses. Given these findings, the World Health Organization recommends using these drugs in pregnancy only after the first trimester and when the benefits of deworming to the health of the mother and unborn fetus outweigh the risks. In anticipation of using mebendazole and albendazole among large pediatric populations in developing countries, the World Health Organization convened an informal consultation on

their use in children under two.³⁷ From this, it was concluded that the incidence of side effects is likely to be the same in this population as in older children and that both agents could be used to treat children as young as 12 months of age using reduced dosages.

Prevention and Control

T. trichiura infection is common in tropical areas, where over 90% of the population is infected in certain communities.³⁸ Most infections are light and asymptomatic. Warm, moist soils in tropical and subtropical regions favor the maintenance of eggs, which can remain alive for months under these optimum conditions. As with *Ascaris* eggs, exposure of *T. trichiura* eggs to direct sunlight for 12 hours or exposure to temperatures over 40 °C for 1 hour kills the embryo inside the egg. Eggs are relatively resistant to chemical disinfectants and can survive in raw or treated sewage for protracted periods. Proper disposal of feces is the primary means of prevention. In areas where untreated human feces are used to fertilize crops, thermophilic composting may be a relatively low-tech approach to destroying these helminth eggs.³⁹

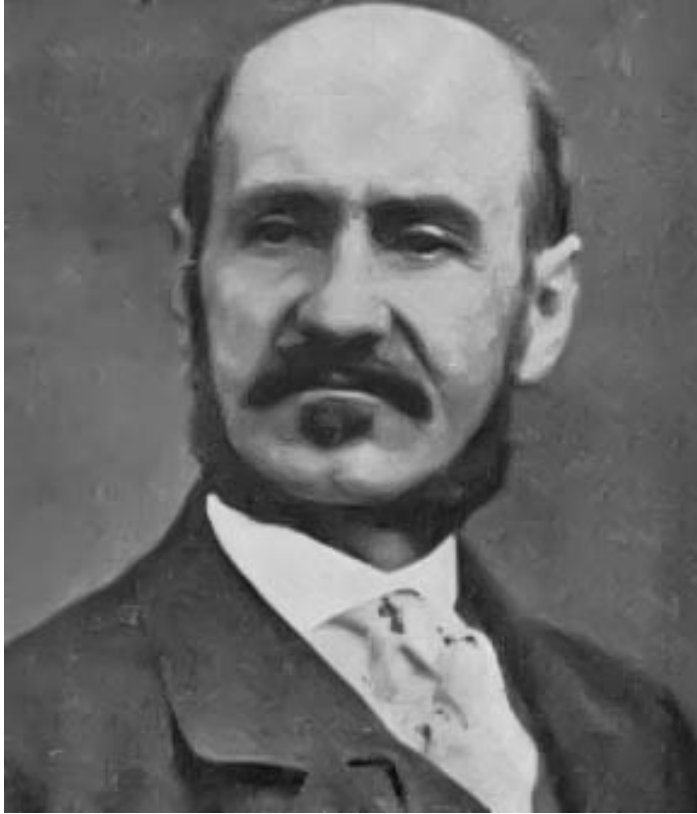
Because school-aged children typically harbor the heaviest *Trichuris* and *Ascaris* infections, and specific anthelmintic chemotherapy with either albendazole or mebendazole can result in catch-up growth and improved cognition for heavily infected individuals, these agents have been used in school-based programs throughout the developing world.^{7, 40, 41} In 2001, the World Health Assembly passed a resolution that recommended its member states administer single dose albendazole and mebendazole on a frequent and periodic basis (1–3 times per year) to control STHs' (*Ascaris*, *Trichuris*, hookworm) morbidity. Because school-aged children contribute the most to *Trichuris* and *Ascaris* transmission in the community, there is also some optimism that widespread treatment could interrupt transmission. High rates of post-treatment STH re-infection require that children must be treated at least annually. The WHO NTD Roadmap for STH elimination by 2030 specifies two indicators: one that measures 75% treatment coverage in endemic countries of preschool and school-aged children and second the proportion of these children receiving regular treatment.⁴²

References

1. Keiser, J.; Utzinger, J., Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *JAMA* **2008**, *299* (16), 1937-48.
2. Bethony, J., et al., Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* **2006**, *367* (9521), 1521-32.
3. Lammie, P. J., et al., A blueprint for success: integration of neglected tropical disease control programmes. *Trends Parasitol* **2006**, *22* (7), 313-21.
4. Webster, J. P., et al., The contribution of mass drug administration to global health: past, present and future. *Philos Trans R Soc Lond B Biol Sci* **2014**, *369* (1645), 20130434.
5. Jourdan, P. M., et al., Soil-transmitted helminth infections. *Lancet* **2018**, *391* (10117), 252-265.
6. Global Burden of Disease Study, C., Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **2015**, *386* (9995), 743-800.
7. Piraja de Silva, M. A., Contribucao para o estudo da schistosomiasena Bahia. *Brazil Med* **1908**, *2* 281-283.

8. Cooper, E. S.; Bundy, D. A., *Trichuris* is not trivial. *Parasitology today (Personal ed.)* **1988**, 4 (11), 301-6.
9. Nokes, C., et al., Moderate to heavy infections of *Trichuris trichiura* affect cognitive function in Jamaican school children. *Parasitology* **1992**, 104 (Pt 3), 539-47.
10. Morgagni, G. B.; Anatomica, X. I. V., Epistolarum anatomicarum duodeviginti ad script pertinentium celeberrimi yin Antonii Marie Valsalvae pars Altera. *Epistola Apud Franciscum Pitheri Venice* **1740**.
11. Cox, F. E., History of human parasitology. *Clin Microbiol Rev* **2002**, 15 (4), 595-612.
12. Bundy, D. A.; Cooper, E. S., *Trichuris* and trichuriasis in humans. *Adv Parasitol* **1989**, 28, 107-73.
13. Roederer, J. G., Nachrichten von der Trichuriden, der Societat der Wissenschaften in Gottingen. *Gottingische Anzeigen von gelebrten Sachen Unter der Aufsicht der Konigliche Gesellschaft der Wissenschaften Part* **1761**, 25, 243-245.
14. Pike, E. H., Bionomics, blood and 51Cr: investigations of *Trichuris muris* and studies with two related species. *Doctoral dissertation Columbia University pp* **1963**, 1-207.
15. Brown, H. W.; West, D., The Whipworm of Man (Seminar). *Merck Sharp and PA* **1954**, 16, 19-22.
16. Belding, D.; New, M., Textbook of Parasitology. *pp* **1965**, 397-398.
17. Brown, H. W., Studies on the rate of development and viability of the eggs of *Ascaris lumbricoides* and *Trichuris trichiura* under field conditions. *Parasitology* **1927**, 14, 1-15.
18. Jackson, J. A., et al., T helper cell type 2 responsiveness predicts future susceptibility to gastrointestinal nematodes in humans. *The Journal of infectious diseases* **2004**, 190 (10), 1804-11.
19. Williams-Blangero, S., et al., Genetic component to susceptibility to *Trichuris trichiura*: evidence from two Asian populations. *Genetic epidemiology* **2002**, 22 (3), 254-64.
20. MacDonald, T. T., et al., Histopathology and immunohistochemistry of the caecum in children with the *Trichuris* dysentery syndrome. *Journal of clinical pathology* **1991**, 44 (3), 194-9.
21. Drake, L., et al., The major secreted product of the whipworm, *Trichuris*, is a pore-forming protein. *Proceedings. Biological sciences / The Royal Society* **1994**, 257 (1350), 255-61.
22. Bennett, A. B., et al., A beta-tubulin gene from *Trichuris trichiura*. *Molecular and biochemical parasitology* **1999**, 103 (1), 111-6.
23. Bundy, D. A.; Cooper, E. S., *Trichuris* and trichuriasis in humans. *Advances in parasitology* **1989**, 28, 107-73.
24. Summers, R. W., et al., *Trichuris suis* therapy in Crohn's disease. *Gut* **2005**, 54 (1), 87-90.
25. Sandborn, W. J., et al., Randomised clinical trial: the safety and tolerability of *Trichuris suis* ova in patients with Crohn's disease. *Aliment Pharmacol Ther* **2013**, 38 (3), 255-63.
26. Reddy, A.; Fried, B., The use of *Trichuris suis* and other helminth therapies to treat Crohn's disease. *Parasitol Res* **2007**, 100 (5), 921-7.
27. Ramanan, D., et al., Helminth infection promotes colonization resistance via type 2 immunity. *Science* **2016**, 352 (6285), 608-12.
28. Hiemstra, I. H., et al., Excreted/secreted *Trichuris suis* products reduce barrier function and suppress inflammatory cytokine production of intestinal epithelial cells. *Mol Immunol* **2014**, 60 (1), 1-7.
29. Ottow, M. K., et al., The helminth *Trichuris suis* suppresses TLR4-induced inflammatory responses in human macrophages. *Genes Immun* **2014**, 15 (7), 477-86.
30. Kapczuk, P., et al., The influence of selected gastrointestinal parasites on apoptosis in intestinal epithelial cells. *Biomolecules* **2020**, 10 (5).
31. Loukas, A., et al., The yin and yang of human soil-transmitted helminth infections. *Int J Parasitol* **2021**, 51 (13-14), 1243-1253.
32. Gilman, R. H., et al., The adverse consequences of heavy *Trichuris* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1983**, 77 (4), 432-8.
33. Joo, J. H., et al., Colonoscopic diagnosis of whipworm infection. *Hepato-gastroenterology* **1998**, 45 (24), 2105-9.
34. Sirivichayakul, C., et al., The effectiveness of 3, 5 or 7 days of albendazole for the treatment of *Trichuris trichiura* infection. *Annals of tropical medicine and parasitology* **2003**, 97 (8), 847-53.
35. Speich, B., et al., Oxantel pamoate-albendazole for *Trichuris trichiura* infection. *N Engl J Med*

- 2014, 370 (7), 610-20.
36. Hotez, P. J., Mass drug administration and integrated control for the world's high-prevalence neglected tropical diseases. *Clin Pharmacol Ther* **2009**, 85 (6), 659-64.
37. Montresor, A., et al., Use of benzimidazoles in children younger than 24 months for the treatment of soil-transmitted helminthiasis. *Acta tropica* **2003**, 86 (2-3), 223-32.
38. Needham, C., et al., Epidemiology of soil-transmitted nematode infections in Ha Nam Province, Vietnam. *Trop Med Int Health* **1998**, 3 (11), 904-12.
39. Kone, D., et al., Helminth eggs inactivation efficiency by faecal sludge dewatering and co-composting in tropical climates. *Water Res* **2007**, 41 (19), 4397-402.
40. Nokes, C., et al., Moderate to high infections of *Trichuris trichiura* and cognitive function in Jamaican school children. *Proc* **1991**, 247, 77-81.
41. Albonico, M., et al., Monitoring drug efficacy and early detection of drug resistance in human soil-transmitted nematodes: a pressing public health agenda for helminth control. *International journal for parasitology* **2004**, 34 (11), 1205-10.
42. Organization, W. H., Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva. Licence: CC BY-NC-SA 3.0 IGO **2020**.



Angelo Dubini, M.D. (1813–1902)

Dubini is credited with describing the adult stage of *Ancylostoma duodenale* (which he later named). He discovered the worm while conducting an autopsy on a young woman. Bilharz, working in Egypt, made the connection between heavy hookworm infection and severe anemia. Some years later, Dubini was called in to help identify the cause of an epidemic of severe anemia and death among workers engaged in digging the 15-kilometer St. Gotthard tunnel, that eventually connected Italy with Switzerland. Dubini identified hookworm as the cause of their illness. He published their combined findings in 1843. This seminal paper was to inspire studies into the cause of “southern laziness”, a disease that gripped the southland following the American Civil War.

18. *Ascaris lumbricoides*

(Linnaeus, 1758)

Pronunciation: \as-kə-rəs\\ləm-bri-kòi-dz\

Introduction

Ascaris lumbricoides (\ASS-ka-ris\\lum-BRI-koy-dz\) is one of the largest nematodes to infect humans. The adult lives in the small intestine, where it can grow to a length of more than 30 cm. *A. lumbricoides* is present in temperate and sub-tropical zones. Current estimates indicate that over one billion people worldwide are infected (CDC, 2023).^{1,3} Infection is via the fecal-oral route, typically through exposure to contaminated food. The most severe consequences of *Ascaris* infection occur in children predisposed to suffer from heavier worm burdens than adults living under similar conditions. *Ascaris* eggs thrive in warm, moist soil and are highly resistant to various environmental conditions. The eggs can survive in sub-arctic regions.⁴ In some developing countries, *Ascaris* eggs are ubiquitous and have been recovered from various environmental



Figure 18.1. Adult female (upper) and male (lower) *Ascaris lumbricoides*. 13–18 cm in length.



Figure 18.2. Adult *Ascaris* in appendix.

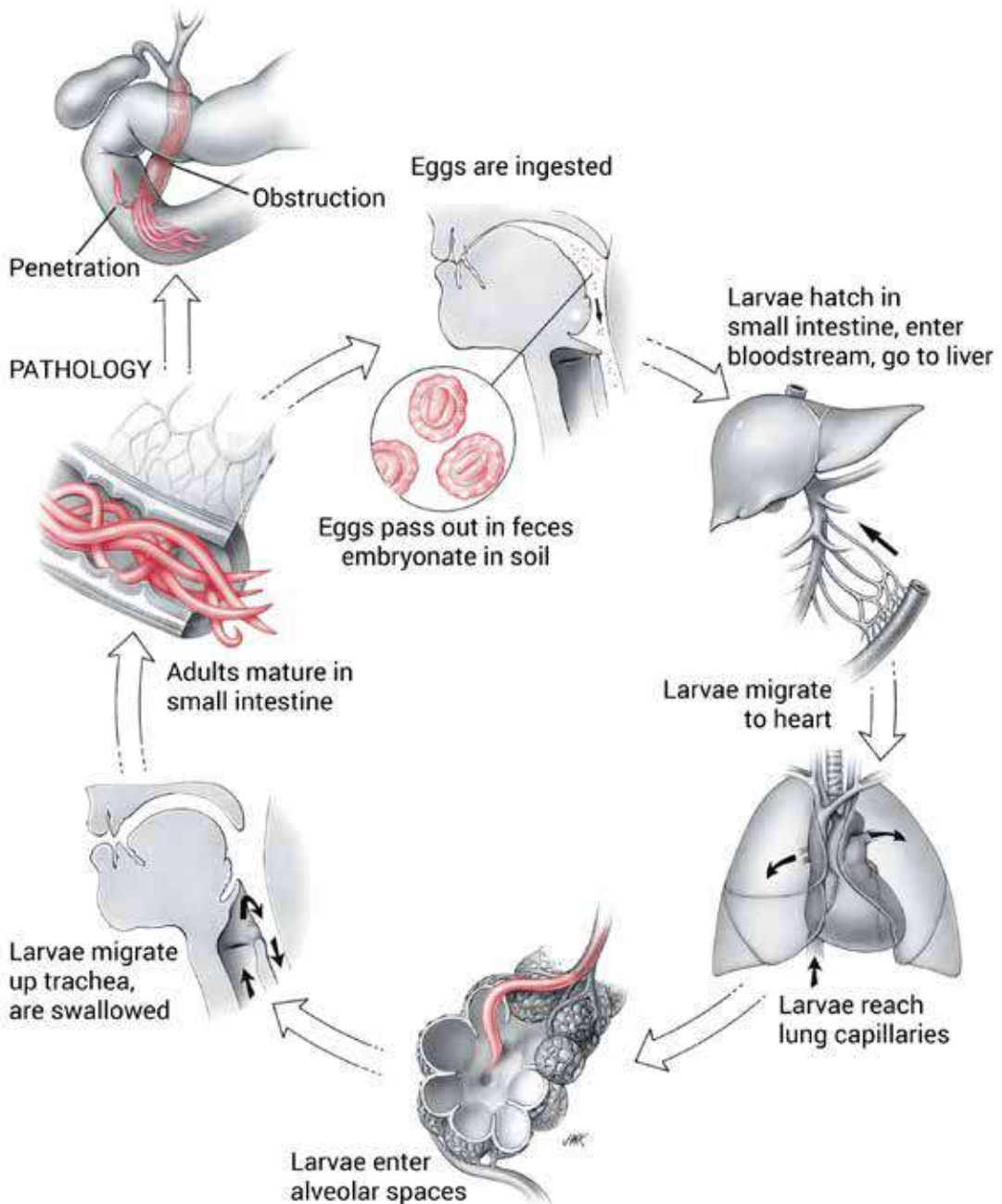
surfaces, including poorly washed hands and even paper currency.^{5,6} The ability of *Ascaris* eggs to survive in these harsh environments accounts for the urban transmission of *Ascaris* in large cities.

In some cases, pigs can serve as a reservoir for *A. lumbricoides*.⁷ It has been suggested that human infection arose coincident with pig domestication, possibly in China.⁸ Molecular epidemiological evidence in Cameroon showed that there is overlap among people and pigs for harboring both species of *Ascaris*, shedding new light on these two closely related nematodes.⁹ If applied to other endemic centers worldwide, this latter approach should provide a more reliable dataset than previous studies which did not apply molecular methods to their investigations.⁸

Historical Information

In 1683, Edward Tyson described the anatomy of *A. lumbricoides*, then known as *Lum-*

Ascaris lumbricoides



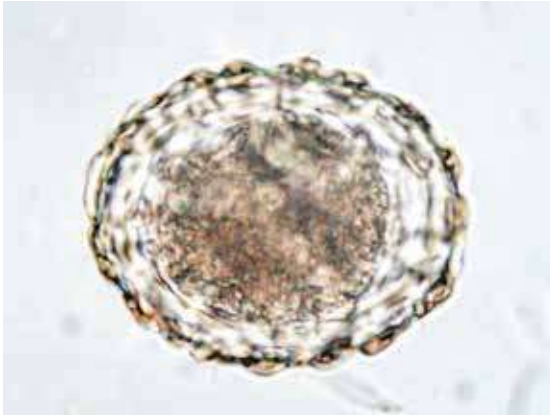


Figure 18.3. Fertilized, unembryonated egg of *A. lumbricoides*. 60 μm x 40 μm .

bricus teres.¹⁰ Carl Linnaeus gave it its current name based on its remarkable similarity to the earthworm, *Lumbricus terrestris*, which he also named.¹⁰ Brayton Ransom accurately described the worm's life cycle.¹¹ In 1922, Shimesu Koino reported on a series of experiments in which he infected himself and his younger brother.¹² For his brother, Koino chose to use *A. suum* eggs, instead of *A. lumbricoides* eggs. The pig ascarid usually fails to complete its life cycle in humans, thus sparing the younger Koino from an overwhelming infection. It is now known that in some cases, *A. suum* in humans can result in the development of adult worms.¹² The older Koino brother consumed 500 *A. lumbricoides* eggs and demonstrated that a pneumonia-like syndrome develops during the early phase of the infection, caused by L3 larvae migrating through the lungs on their way to the stomach. The older Koino became seriously ill, but did not suffer permanent disability.

Life Cycle

The adult worms (Figs. 18.1, 18.2) live freely in the lumen of the upper small intestine, where they derive all their nutrition from our predigested food, chyme, and cellular debris. The worms maintain themselves in the lumen of the small intestine by assuming an S-shaped configuration, pressing their cuticular sur-

faces against the columnar epithelium of the intestine, and continually moving against the flow of peristalsis. The worms are covered with a tough, thick cuticle composed of collagens and unusual lipids, enabling them to resist being digested by host hydrolases. The adult worms produce a battery of protease inhibitors, some of which interfere with host digestion.

The daily egg production of the adult female worm is staggering, producing approximately 200,000, each of which can survive up to 10 years in the external environment under favorable conditions.¹³ Since adult worms can live for up to five years, this represents an astounding level of fecundity. The uterus of the adult female worm may contain up to 27 million eggs at any one time. To synthesize that amount of sterol necessary for massive egg production, *Ascaris* possesses a special biochemical pathway to carry out this oxygen-dependent reaction in the low-oxygen folds of the small intestine. It assembles the components of the reaction on a special oxygen-avid hemoglobin.¹⁴ Since *Ascaris* is probably an obligate anaerobe, its hemoglobin might also detoxify its environment by removing oxygen through a unique chemical coupling with NO.^{15, 16}

Fertilized eggs (Fig. 18.3) pass out of the adult but are not yet embryonated. Eggs become incorporated into the fecal mass and exit the host in feces. As a soil-transmitted helminth (STH), *Ascaris* embryonation occurs in the soil and is completed within 2–4 weeks. Eggs not reaching soil immediately (e.g., municipal sewage sludge) can survive in moist environments for up to 2 months.¹⁷ A unique lipoprotein, known as ascaroside, occupies a portion of the egg's inner layer and may confer some of the environmental resistance properties attributed to *Ascaris* eggs. A second mucopolysaccharide component on the ova's surface provides adhesive properties, allowing them to accumulate on

various environmental surfaces. The embryonated egg is the infective stage and must be swallowed for the life cycle to continue. The L1 larva develops into the L2 larva inside the egg, and the worm retains the L2 cuticle around its body.

In the host, the L2 larva develops into the L3 larva while still inside the egg and enclosed by the cuticle.¹⁸ When the egg reaches the small intestine, solubilization of certain outer layers of the eggshell is mediated by bile salts. This allows the embryo to receive environmental cues that stimulate it to hatch. An egg protease is activated by alkaline conditions, ensuring it is in the right anatomic location inside the host to continue its journey. The infectious process is accompanied by a dramatic shift in *Ascaris* metabolism from aerobic to anaerobic.¹⁹

The immature L3 parasite, now in the small intestinal lumen, penetrates the intestinal wall, enters the lamina propria, penetrates a capillary, and is carried by the portal circulation to the liver. In the liver, the worm feeds on parenchymal tissue (*foie gras d'homme*) and grows (Fig. 18.4). It then migrates via the bloodstream to the heart and eventually into the pulmonary circulation. The larva molts once more and grows larger in length and in diameter. It becomes stuck in an alveolar capillary since its diameter is now much greater

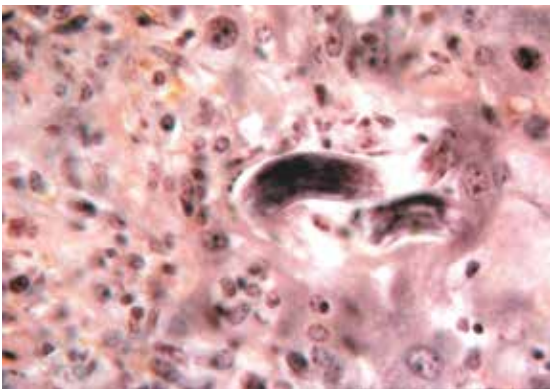


Figure 18.4. Larvae of *A. lumbricoides* in liver of experimentally infected mouse.



Figure 18.5. Larva of *A. lumbricoides* in lung of an experimentally infected mouse.

than that of the vessel. The worm receives a thigmotactic (touch) signal, initiating a behavior that results in its breaking out into the alveolar spaces (Fig. 18.5). This is the phase of the infection that caused Koino to experience his “*verminous*” pneumonia.

The larva migrates up the bronchi into the trachea and across the epiglottis; it is swallowed, finally reaching the lumen of the small intestine for a second time. After an additional molt, the worms grow prodigiously, maturing to adulthood in about six weeks. Adult worms then mate. Occasionally, egg production may precede mating. When this occurs, the female worm releases infertile eggs. Rarely a single female worm is acquired, again resulting in infertile egg production.

Cellular and Molecular Pathogenesis

The most intense host reactions occur during the migratory phase of infection. *Ascaris* antigens released during the molting process have allergenic properties, causing inflammation associated with eosinophilic infiltration of the tissues, peripheral eosinophilia, and antibody responses, increasing serum IgE levels. At least one of these allergens is known as *Ascaris* body fluid allergen (ABA-1). It has been suggested that IgE responses

to ABA-1 and related antigens confer resistance to *Ascaris* infections.²⁰

The links between IgE levels, eosinophilia and *Ascaris* infections have given rise to several hypotheses to explain the impact of helminth infections, such as ascariasis, on the atopic state of the host. Among them is that atopy evolved as an adaptive mechanism to promote host resistance to helminths.²¹ During migration, *Ascaris* larvae trigger allergic responses in the lungs that resemble the pathogenesis of asthma.²² Both processes may be linked to polymorphisms of the β -2-adrenoreceptor gene.²³ In addition, *Ascaris* adults secrete an anti-trypsin factor, enabling ingestion of a portion of any meal before the host absorbs it. In-depth proteomic studies of the excretory/secretory products produced by *A. lumbricoides* and *A. suum* are not only helping to improve our understanding of how this helminth influences the host but may also serve as targets for vaccine development.²⁴

School-aged children are predisposed to heavy infections with *Ascaris*, although the reason for this remains unclear. Many of the same children also harbor large numbers of *Trichuris trichiura*. Such children may suffer from impairments in their physical growth and cognitive and intellectual development.²⁵ It has been hypothesized that *Ascaris* interferes with host nutrition, possibly through competition for nutrients, but this hypothesis is, as yet, unproven. Other studies indicate that children harboring *Ascaris* can develop fat, protein, and vitamin A malabsorption, lactose intolerance from damaged intestinal mucosa, impaired intestinal permeability, and anorexia.²⁶ It has been further hypothesized that chronic intestinal inflammation leads to anorexia and cachexia, although there is no strong evidence. Some longitudinal studies in Asia and Africa have compared the growth of *Ascaris*-infected children to that of children given antihelminthics, with most studies

showing a significant improvement in weight after treatment.²⁷ In a few of these studies, treated children also had a greater increase in height compared to those untreated. The effects on growth were more pronounced in children with the heaviest infections. Additional studies also suggest that *Ascaris* may impair mental processing.²⁸

Clinical Disease

Migratory Phase

The intensity of the systemic response to the larva of *Ascaris* correlates directly with the number of worms migrating at any one time. If infection is light, and only a few parasites traverse the tissues, the host response is negligible, and infected individuals remain asymptomatic. In heavy infections, following ingestion of hundreds to thousands of eggs, the patient can experience intense pneumonitis, enlargement of the liver, and generalized toxicity that may last up to two weeks. The pneumonitis, known as Löeffler's syndrome, presents with eosinophilic infiltrates, an elevated IgE, and bronchospasm that clinically resembles asthma. Similar phenomena have also been described among uninfected laboratory workers who develop bronchospasm after previous sensitization to *Ascaris* allergens.²¹

Intestinal Phase

Although adult worms in the intestine usually cause few symptoms, when they are numerous, their sheer bulk may cause fullness and even obstruction. Adult worms migrate when irritated (high fevers, drugs, etc.), which may lead them to penetrate the liver, obstruct the biliary tract, or perforate the intestine and facilitate peritonitis. Individuals with mild and moderate infections are rarely symptomatic. Most commonly, these individuals become aware of the infection by examining the stools for another reason: because of the passage of an adult worm in the stool

or by regurgitating it during an episode of vomiting. Heavy infections may lead to a large bolus of adults obstructing the intestinal lumen, especially the ileum (Figs. 18.6, 18.7). In developing countries throughout the tropics, acute *Ascaris* intestinal obstruction is a leading cause of a “surgical abdomen” in children, accounting for up to 35% of all intestinal obstruction in these regions, and 10,000 deaths annually.^{28, 29}

Hepatobiliary Ascariasis (HPA)

Adult worms may migrate into the biliary tree, causing hepatobiliary and pancreatic ascariasis. This problem occurs more commonly in small children who harbor large numbers of worms. Migration of adult worms into the hepatobiliary tree can lead to cholecystitis, cholangitis, hepatic abscess, pancreatitis, and death may ensue.^{30, 31} An ultrasound screen of the general population in Kashmir,



Figure 18.6. Child with distended abdomen due to large bolus of *A. lumbricoides* adult worms in small intestines.



Figure 18.7. Adult *Ascaris* recovered from child in Fig. 18.6. after treatment with mebendazole.

India, determined that 0.5% of the adults in this region had evidence of hepatobiliary ascariasis.³¹

Neonatal Ascariasis

Neonatal ascariasis may occur when *Ascaris* larvae enter the placenta.^{32, 33} Although transplacental transmission is common among animal ascarids, the true extent of this phenomenon among humans is unknown.

Diagnosis (see Clinical Appendix)

A. lumbricoides infection cannot be specifically diagnosed solely based on signs or symptoms during the migratory or intestinal phases of the infection. Hepatobiliary ascariasis is difficult to diagnose by conventional radiographic techniques, as the worms often move out of the bile or pancreatic duct after eliciting symptoms. In some endemic areas, ultrasonography and endoscopic retrograde cholangiopancreatography (ERCP) have been used diagnostically.^{30, 31, 34} The clinical suspicion of infection with intestinal helminths is the usual reason to request a stool examination.

Ascaris eggs (Figs. 18.3, C.39) are easily recognized on stool examination. If only a few eggs are present, they may be missed but can be identified if the stool specimen is

concentrated by several standard techniques (Appendix C). Since so many eggs are passed each day by individual female worms the likelihood of finding them, even in patients with light infections, is high. The presence of infertile *Ascaris* eggs is diagnostically significant, as even a single female worm may have serious clinical consequences if she migrates. Defective eggs missing the outer mammillations (nipple-like projections) are occasionally observed. Serological tests to detect antibodies to *A. lumbricoides* are available but are generally only used in epidemiological studies rather than clinically due to concerns with cost and specificity.³⁵⁻³⁷ Molecular antigen tests are not widely available for helminth infections including for *A. lumbricoides* and quality control and inter-laboratory variability needs further work.³⁸

Treatment (see Clinical Appendix)

Albendazole and mebendazole are the treatments of choice for ascariasis.^{39, 40} For school-based deworming programs, a single dose of albendazole 400 mg or mebendazole 500 mg is usually effective. The older drug pyrantel pamoate is also effective. Piperazine citrate can be used in cases of intestinal obstruction because it paralyzes the worm's myoneural junctions, allowing them to be expelled by peristalsis. However, this drug is no longer widely available. The migratory parenteral phase of the infection is transitory, seldom diagnosed, and not typically treated. If infection is heavy, a pneumonia-like syndrome may alert the physician, and patients may be treated symptomatically with corticosteroids.⁴¹

Surgical intervention is sometimes necessary if many worms result in an intestinal or biliary obstruction. These conditions often pres-

ent as a medical emergency due to anaerobic necrosis of intestinal tissue. In some cases, the adult worms can be removed endoscopically.

Prevention and Control

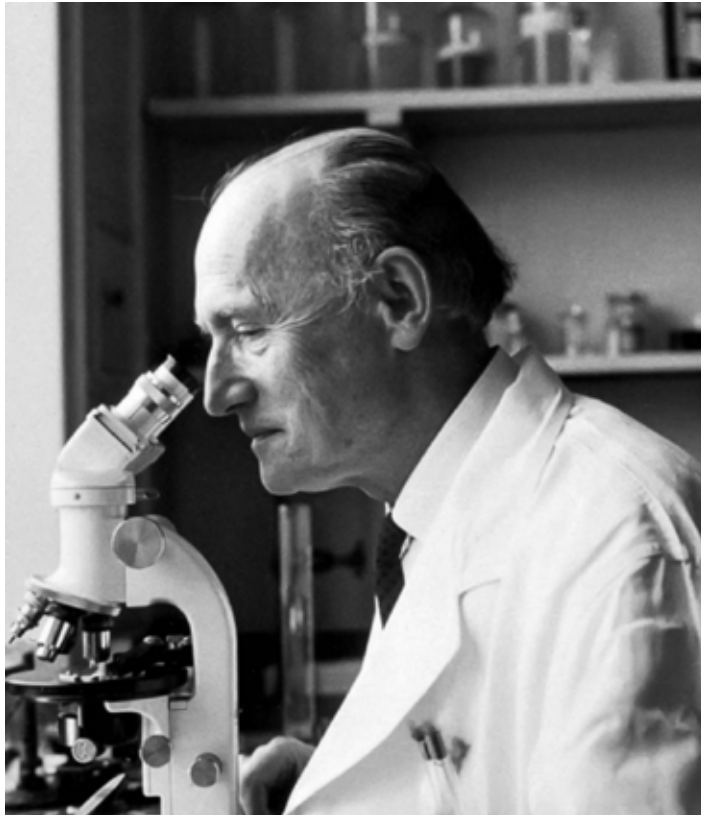
A. lumbricoides is present in temperate and sub-tropical zones. Its highest prevalence is in tropical, rural areas where sanitation is absent. *Ascaris* worms are hardy, and it is not uncommon to find ascariasis cases in urban slums. In some regions of Africa, 95% of the population is infected, and in parts of Central and South America, 45% are infected. In the United States, infection was once prevalent in southern, rural communities, but no more recent studies have been conducted.^{41, 42} Sex or race is of no epidemiological consequence in the distribution of ascariasis. Although persons of all ages are susceptible, the infection predominates among school-aged children, who typically harbor the highest intensity of infections. This observation and the health and educational benefits of deworming led the 2001 World Health Assembly to recommend using single-dose treatments of children with albendazole or mebendazole as a cornerstone of a global deworming program. Through such mass drug administration programs, the global prevalence of ascariasis is believed to have diminished by approximately 25% over the last two decades.³

Ascaris eggs are destroyed by exposure to direct sunlight for 12 hours and die at temperatures over 40 °C. Exposure to cold does not adversely affect the eggs. They have been known to survive the ordinary freezing temperatures of winter months in the temperate zones. The eggs are also resistant to many commonly used chemical disinfectants and can thrive in treated sewage for months to years.

References

- DeSilva, N. R.; Brooker, S., Soil-transmitted helminth infections: updating the global picture. *Trends Parasitol* **2003**, *19* 547-51.
- Hotez, P. J., et al., Helminth infections: the great neglected tropical diseases. *J Clin Invest* **2008**, *118* (4), 1311-21.
- Global Burden of Disease Study, C., Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **2015**, *386* (9995), 743-800.
- Embil, J. A., et al., Prevalence of *Ascaris lumbricoides* infection in a small Nova Scotian community. *The American journal of tropical medicine and hygiene* **1984**, *33* (4), 595-8.
- Jeandron, A., et al., A quantitative assessment method for *Ascaris* eggs on hands. *PloS one* **2014**, *9* (5), e96731.
- Saturnino, A. C., et al., [Transmission of enteroparasitosis through currency notes]. *Acta Cir Bras* **2005**, *20 Suppl 1*, 262-5.
- Cavallero, S., et al., Genotyping of *Ascaris* spp. infecting humans and pigs in Italy, Slovakia and Colombia. *Infect Genet Evol* **2021**, *94*, 104997.
- Peng, W., et al., Genetic variation in sympatric *Ascaris* populations from humans and pigs in China. *Parasitology* **1998**, *117* (Pt 4), 355-61.
- Nkouayep, V. R., et al., Molecular epidemiology of *Ascaris* species recovered from humans and pigs in Cameroon. *Trans R Soc Trop Med Hyg* **2022**, *116* (10), 949-958.
- Tyson, E., *Lumbricus teres*, or some anatomical observations on the roundworm bred on the human bodies. *Philos Trans* **1683**, *13*, 153-161.
- Ransom, R. H., et al., Life history of *Ascaris lumbricoides* and related forms. *Res* **1917**, *11*, 395-398.
- Koino, S., Experimental infections on human body with ascarides. *Jpn Med World* **1922**, *2*, 317-320.
- Khuroo, M. S., Ascariasis. *Gastroenterol Clin North Am* **1996**, *25* (3), 553-77.
- Sherman, D. R., et al., Components of sterol biosynthesis assembled on the oxygen-avid hemoglobin of *Ascaris*. *Science (New York, N.Y.)* **1992**, *258* (5090), 1930-2.
- Minning, D. M., et al., *Ascaris* haemoglobin is a nitric oxide-activated 'deoxygenase'. *Nature* **1999**, *401* (6752), 497-502.
- Goldberg, D. E., Oxygen-Avid Hemoglobin of *Ascaris*. *Chemical reviews* **1999**, *99* (12), 3371-3378.
- Bryan, F. D.; J., Diseases transmitted by foods contaminated by waste water. *Protein* **1977**, *40* 45-56.
- Geenen, P. L., et al., The morphogenesis of *Ascaris suum* to the infective third-stage larvae within the egg. *J Parasitol* **1999**, *85* (4), 616-22.
- Harmych, S., et al., Role of dihydrolipoyl dehydrogenase (E3) and a novel E3-binding protein in the NADH sensitivity of the pyruvate dehydrogenase complex from anaerobic mitochondria of the parasitic nematode, *Ascaris suum*. *Molecular and biochemical parasitology* **2002**, *125* (1-2), 135-46.
- McSharry, C., et al., Natural immunity to *Ascaris lumbricoides* associated with immunoglobulin E antibody to ABA-1 allergen and inflammation indicators in children. *Infection and immunity* **1999**, *67* (2), 484-9.
- Lynch, N. R., et al., Relationship between helminthic infection and IgE response in atopic and nonatopic children in a tropical environment. *The Journal of allergy and clinical immunology* **1998**, *101* (2 Pt 1), 217-21.
- Buendia, E., et al., The IgE response to *Ascaris* molecular components is associated with clinical indicators of asthma severity. *World Allergy Organ J* **2015**, *8* (1), 8.
- Ramsay, C. E., et al., Association of polymorphisms in the beta2-adrenoreceptor gene with

- higher levels of parasitic infection. *Human genetics* **1999**, 104 (3), 269-74.
24. Wang, T., et al., Proteomic analysis of the excretory-secretory products from larval stages of *Ascaris suum* reveals high abundance of glycosyl hydrolases. *PLoS Negl Trop Dis* **2013**, 7 (10), e2467.
 25. Hadidjaja, P., et al., The effect of intervention methods on nutritional status and cognitive function of primary school children infected with *Ascaris lumbricoides*. *The American journal of tropical medicine and hygiene* **1998**, 59 (5), 791-5.
 26. Crompton, D. W. T.; Nesheim, M. C., Nutritional impact of intestinal helminthiasis during the human life cycle. *Annual review of nutrition* **2002**, 22, 35-59.
 27. O'Lorcain, P.; Holland, C. V., The public health importance of *Ascaris lumbricoides*. *Parasitology* **2000**, 121, S51-71.
 28. Crompton, D. W., *Ascaris* and ascariasis. *Advances in parasitology* **2001**, 48, 285-375.
 29. Andrade, A. M., et al., Intestinal Obstruction in a 3-Year-Old Girl by *Ascaris lumbricoides* Infestation: Case Report and Review of the Literature. *Medicine (Baltimore)* **2015**, 94 (16), e655.
 30. Khuroo, M. S., et al., Hepatobiliary and pancreatic ascariasis in India. *Lancet (London, England)* **1990**, 335 (8704), 1503-6.
 31. Khuroo, M. S., Hepatobiliary and pancreatic ascariasis. *Indian journal of gastroenterology : official journal of the Indian Society of Gastroenterology* **2001**, 20 Suppl 1, C28-32.
 32. Costa-Macedo, L. M. D.; Rey, L., *Ascaris lumbricoides* in neonate: evidence of congenital transmission of intestinal nematodes. *Rev Inst Med Trop Sao Paulo* **1990**, 32, 351-354.
 33. Synovia, C. B., et al., Fetal response to maternal ascariasis as evidenced by anti-*Ascaris lumbricoides* IgM antibodies in the cord blood. *Acta Paediatr Scand* **1991**, 80, 1134-1138.
 34. Schulman, A., Ultrasound appearances of intra- and extrahepatic biliary ascariasis. *Abdominal imaging* **1998**, 23 (1), 60-6.
 35. Bhattacharyya, T., et al., Possible approach for serodiagnosis of ascariasis by evaluation of immunoglobulin G4 response using *Ascaris lumbricoides* somatic antigen. *J Clin Microbiol* **2001**, 39 (8), 2991-4.
 36. Santra, A., et al., Serodiagnosis of ascariasis with specific IgG4 antibody and its use in an epidemiological study. *Trans R Soc Trop Med Hyg* **2001**, 95 (3), 289-92.
 37. Lamberton, P. H.; Jourdan, P. M., Human Ascariasis: Diagnostics Update. *Curr Trop Med Rep* **2015**, 2 (4), 189-200.
 38. Leles, D., et al., ITS1 intra-individual variability of *Ascaris* isolates from Brazil. *Parasitol Int* **2010**, 59 (1), 93-6.
 39. Aubry, M. L., et al., Aspects of the pharmacology of a new anthelmintic: pyrantel. *British journal of pharmacology* **1970**, 38 (2), 332-44.
 40. Chevarria, A. P., et al., Mebendazole, an effective broad spectrum anthelminthic. *Am Med Hyg* **1973**, 22 592-595.
 41. Blumenthal, D. S.; Schultz, M. G., Incidence of intestinal obstruction in children infected with *Ascaris lumbricoides*. *The American journal of tropical medicine and hygiene* **1975**, 24 (5), 801-5.
 42. Starr, M. C.; Montgomery, S. P., Soil-transmitted Helminthiasis in the United States: a systematic review--1940-2010. *Am J Trop Med Hyg* **2011**, 85 (4), 680-4.



Percy Cyril Claude Garnham, M.D. (1901–1994)

Garnham was a generalist, conducting laboratory and field-based research on leishmaniasis, toxoplasmosis, and malaria. Working with Henry Shortt, and a host of other colleagues, the group discovered the hypnozoite, the stage responsible for relapses of infection with *Plasmodium vivax* and *P. ovale*. Garnham also produced a wealth of publications on non-primate and non-human primate malarias, in addition to those infecting humans. His book, *Malaria Parasites and Other Haemosporidia*, remains a classic in the field of malariology.

19. The Hookworms

Necator americanus

(Stiles 1902)

Pronunciation: \nə-'kāt-ər\

Ancylostoma duodenale

(Dubini 1843)

Pronunciation: \an-sə-'lās-tə-mə\

Introduction

Two species of hookworm account for most human infections; *Necator americanus* (ne-KAY-tor\) and *Ancylostoma duodenale* (an-see-LOS-tow-mah\).^{1,2} *N. americanus* is the more common human hookworm (especially on the African Continent and Latin America) except in some focal areas of Egypt, India, and China. A third species, *Ancylostoma ceylanicum* (SCI-la-nee-cum\), is also found as a human parasite in Southeast Asia.³ Adult hookworms inhabit the small intestine and feed on intestinal villi and blood. Blood loss resulting from adult hookworms in the intestine leads to protein and iron deficiency and anemia. Hookworms infect approximately 200 million people in the developing nations of the tropics, making this one of the most prevalent human infections worldwide and one of the most common causes of iron-

deficiency anemia.^{4,7} According to some estimates, hookworm ranks with schistosomiasis as the leading helminth infection in terms of deaths and disability-adjusted life years (DALYs) lost more than any other human helminth infection.^{1,8,9}

Children heavily infected with hookworms are likely to develop deficits in both physical and cognitive development and are more susceptible to other intercurrent infections.^{1,8,10,11} Hookworm is an important health threat for women of reproductive age. More than 40 million pregnant women are estimated to be infected with hookworms in endemic countries.⁵ The resulting iron deficiency and malnutrition during pregnancy adversely affect intrauterine growth, birth weight, and maternal survival.¹¹ Hookworm also increases the likelihood of premature birth and may contribute to maternal mortality.¹²

The distribution of the two species was thought to be discrete, but both species have been shown to occupy at least some of the same regions of Africa, South America, and Asia. *N. americanus* is the predominant hookworm worldwide, with the highest rates in Sub-Saharan Africa, tropical regions of the Americas, China, and Southeast Asia.¹ *A. duodenale* is more focally endemic in parts of China, India, North Africa, Sub-Saharan Africa, and a few regions of the Americas. *N. americanus* infection still occurs in focal areas of poverty in the Southern United States.¹³ There are no known reservoirs for *A. duodenale* or *N. americanus*. Because of the thermotolerance of hookworm larvae in the soil, due to climate change and global warming human hookworm infection may emerge as the predominant helminthic infection on the African Continent.¹⁴

A. ceylanicum, is found mainly in dogs,¹⁵ and cats, but infects humans living in Malaysia, and elsewhere in Asia.¹⁶⁻²⁰ The dog is the primary host for *A. caninum*, but it has not been

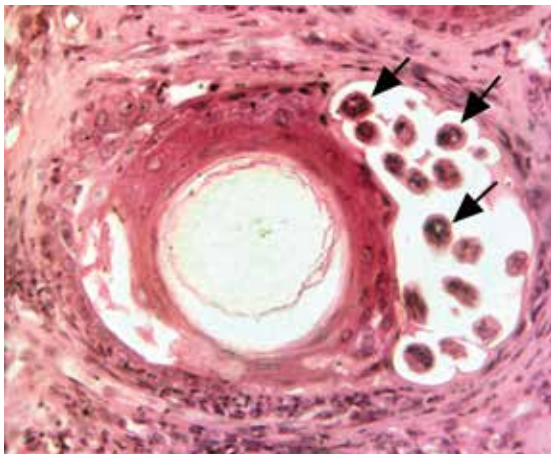
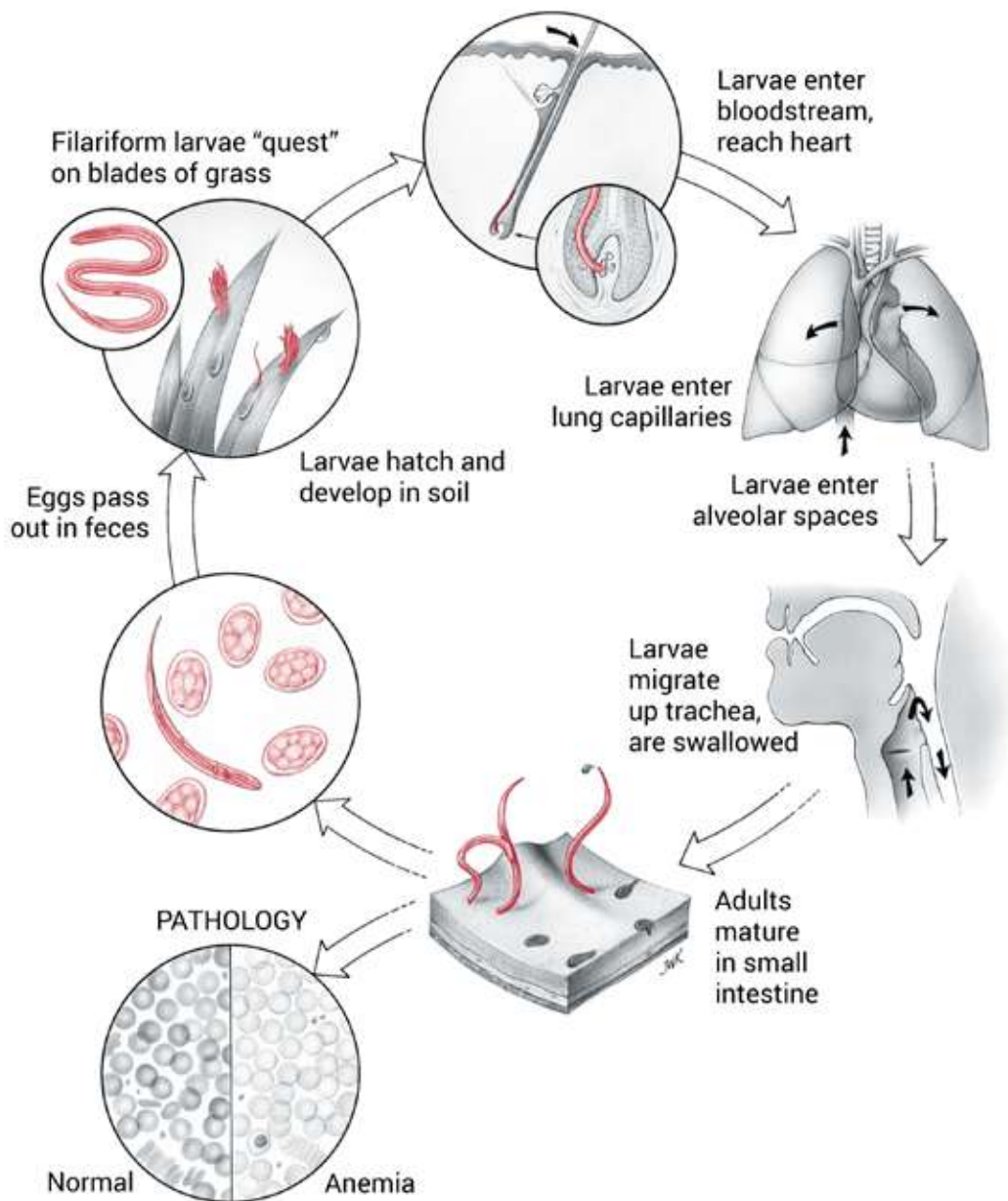


Figure 19.1. Hookworm larvae (arrows) in skin of experimentally infected dog.

Necator americanus

well established whether this hookworm causes mature human infection, save for a few reported cases in Australia where it has been implicated as the cause of eosinophilic enteritis syndrome.²¹ *A. braziliense*, whose definitive hosts are dogs and cats, causes an aberrant infection known as *cutaneous larva migrans*.²²

Historical Information

N. americanus likely originated in Asia, while *A. duodenale* most likely came from Africa.²³ Hookworms have been infecting humans for thousands of years, while controversy exists as to whether hookworm was present in the Americas before European exploration and colonization.^{23, 24} Angelo Dubini first reported human infection with *A. duodenale* in 1843. Arthur Looss, working in Egypt, demonstrated percutaneous transmission of hookworm infection and described the basic features of its life cycle.²⁵ Gerald Schad further defined the biology of hookworms, demonstrating the ability of *A. duodenale* larvae to remain in a dormant arrested state in human tissues.²⁶

In the past, hookworm infection was common in the United States, primarily in the rural South and in Puerto Rico.^{27, 28} Because the disease it caused, iron deficiency anemia, was thought to be a major obstacle to the economic development in the South following the Civil War, John D. Rockefeller, Sr. established the Rockefeller Sanitary Commission (which later became the Rockefeller Foundation) in 1909, for the sole purpose of eliminating hookworm from the United States and Puerto Rico.²⁸ In 1902, Charles W. Stiles first described *N. americanus*, and was largely responsible for convincing Frederick Gates, a Baptist minister and Rockefeller's key advisor, to establish the Commission.²⁹

The prevalence of hookworm infection in the United States has been reduced almost

to the point of elimination, but this resulted less from any planned intervention than from the general improvement in socioeconomic conditions. Economic development also accounted for controlling malaria and typhoid fever in the United States. Much of our knowledge regarding the natural history and pathogenesis of hookworm infection was based on the work of investigators funded by the Rockefeller philanthropies, including William Cort, Auriel O. Foster, Asa C. Chandler, J. Allen Scott, and Norman Stoll. Stoll described hookworm as “the great infection of mankind.”³⁰

Life Cycle

Hookworms are soil-transmitted helminths (STHs). Infection begins when the L3 (filariform) larvae actively penetrate the cutaneous tissues, usually through a hair follicle (Fig. 19.1) or an abraded skin area. The release of hydrolytic enzymes may facilitate skin invasion. Once in the subcutaneous tissues, larvae enter capillaries and are carried passively through the bloodstream to the lungs' capillaries. The L3 larvae break out of the alveolar capillaries and complete its migratory phase by crawling up the bronchi and trachea, over



Figure 19.2. Adult female *Ancylostoma duodenale*. 10 mm.



Figure 19.3. Adult male *A. duodenale*. Note hand-like bursa at tail end. 8 mm.

the epiglottis, and into the pharynx, a pathway similar to the one taken by the L3 larvae of *A. lumbricoides* and *Strongyloides stercoralis*. The larvae are swallowed and proceed into the stomach. Two molts occur in the small intestine, resulting in the development of an adult worm (Fig. 19.2, 19.3).

A. duodenale larvae are also infective when swallowed³¹, and in some regions, oral ingestion may be the predominant transmission mode. Larvae that infect orally may undergo two molts to adulthood without leaving the gastrointestinal (GI) tract, and a syndrome known as Wakana disease, characterized by nausea, vomiting, cough, and difficulty breathing, can develop.³²

Forty days following maturation and copulation, the female worms begin laying eggs (Fig. 19.4), completing the life cycle. In some infections with *A. duodenale*, larvae may stay longer in tissues before transiting to the intestine, resulting in delayed egg production.³³ Adult *A. duodenale* worms live an average of one year. *Necator americanus* can live up to 3-5 years³⁴; this species' maximum recorded survival time is 15 years.³⁵

In endemic areas, where reinfections are continual, the development of some L3 larvae of *A. duodenale* (but not *N. americanus*) is

delayed. After entering the host, these larvae penetrate into bundles of skeletal muscles and become dormant. They can later resume their development and complete the life cycle.²⁶ Larval arrested development in human tissues occurs during times of the year when the external environmental conditions are unfavorable to parasite development in the soil. Larval arrest also occurs during pregnancy, and development resumes at the onset of parturition. Vertical transmission of *A. duodenale* infection in neonates has been reported when larvae migrate into breast milk.³⁶

The adult worms feed on intestinal villi and blood in the small intestine (Fig. 19.5). Morphologically, each species can be differentiated based on the mouthparts of the adults. *A. duodenale* possesses cutting teeth (Fig. 19.6), whereas *N. americanus* has rounded cutting plates (Fig. 19.7). The adult male hookworms are differentiated from the females by an elaborate copulatory bursa.

A. duodenale and *N. americanus* exhibit major differences in their life cycles and pathogenicity. *A. duodenale* is generally considered the more virulent of the two species because it is larger, causes more blood loss, produces more eggs, and has several modes of transmission other than penetrating skin.^{1,2} The female passes eggs into the lumen of the



Figure 19.4. Fertilized, embryonated hookworm egg. 65 μm x 40 μm .



Figure 19.5. Hookworm adult, diagnosed by endoscopy.

small intestine. *A. duodenale* produces about 28,000 eggs per day, and *N. americanus* about 10,000. The eggs embryonate to the four-cell and eight-cell stages immediately after they pass into the external environment along with the fecal mass. In warm, moist, sandy, or loamy soils, the embryo develops into the L1 (rhabditiform) larva within 48 hours of deposition. After hatching, the larva feeds on fecal matter in the immediate surroundings, grows, then molts twice to develop into the infective L3 larva. Filariform larvae do not feed, and are generally considered to be in

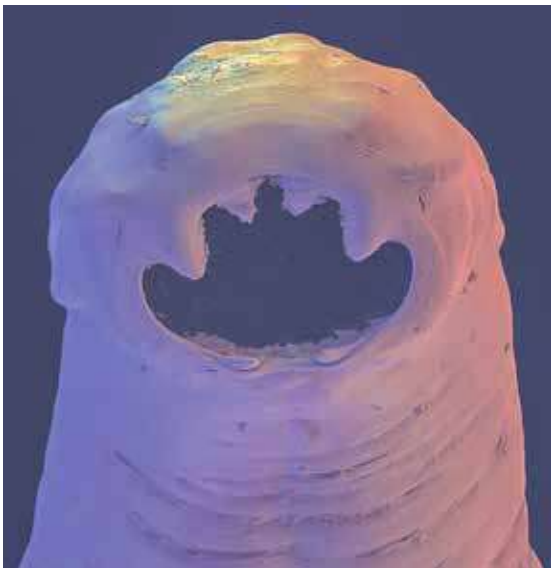


Figure 19.6. Scanning EM of head of *Ancylostoma duodenale*. Note teeth. Photo by D. Scharf.



Figure 19.7. Scanning EM of head of *Necator americanus*. Note cutting plates. Photo by D. Scharf.

a developmentally arrested state.³⁷ The L3 larvae actively seek out the highest point in the environment (e.g., the tops of grass blades, small rocks), where they are more likely to come into direct contact with human skin. This activity is known as “questing.” In endemic areas, it is common for many L3 larvae to aggregate on dewy grass, increasing the chances for multiple infections of the same host. Sandy/loamy soils are particularly favorable for hookworm larval survival and transmission.

Cellular and Molecular Pathogenesis

The L3 hookworm larvae penetrate unbroken skin with secreted enzymes, including a metalloprotease and a family of cysteine-rich secretory proteins known as the *Ancylostoma* secreted proteins.^{38, 39} Repeated infection results in immediate hypersensitivity and other inflammatory responses comprising hookworm dermatitis. Subsequent larval migration through the lungs may result in pulmonary inflammation, resulting in pneumonitis.

Most of the pathology of hookworm infection results from the presence of adult hookworms in the small intestine. The adult worms derive their nourishment from eating villous tissue. They also suck blood directly from their attachment site to the intestinal mucosa and submucosa. Adult worms possess a well-developed esophageal bulb, enabling them to pump blood from the capillary bed of the mucosa. Adult parasites secrete an anticoagulant that blocks the action of host factor Xa and the VIIa tissue factor complex.^{40, 41} Hookworms also secrete a protein that functions as a platelet inhibitor through blockade of GPIIb/IIIa.⁴²⁻⁴⁴ Blood loss continues for about three days after the worms move to a new location through the combined activity of these products. Each *A. duodenale* adult sucks 0.1–0.2 ml of blood daily, while each *N. americanus* adult worm sucks 0.01–0.02 ml of blood daily.⁴⁵ Following blood ingestion, the adult hookworms rupture the red blood cells with hemolysins and then break down host hemoglobin in an ordered manner employing hemoglobin-specific proteases.^{46,47} A unique Nu class of glutathione S-transferase helps the parasite detoxify the heme released from hemoglobin digestion.⁴⁸ In addition to blood loss, protein loss can contribute to protein malnutrition, a problem that persists in low socioeconomic conditions where hookworm infection remains endemic.⁴⁹ Production of a hookworm serine protease inhibitor that interferes with pancreatic enzyme-based digestion may also interfere with the breakdown of ingested foods, leading to malabsorption and consequent retardation of childhood growth and development.⁵⁰

Repeated exposure to hookworm infection does not necessarily result in protective immunity.⁵¹ Although a humoral antibody response to numerous hookworm antigens can be demonstrated in naturally infected individuals, the presence of specific antibod-

ies often does not correlate with resistance to infection.⁵² Some immunoglobulins may even serve as a marker for hookworm infection.⁵³ The absence of effective immunity may explain why the intensity of hookworm infection often increases with age in endemic regions, in contrast to the other major STH infections (e.g., *Ascaris* and *Trichuris*), for which the intensity peaks in childhood.⁸

A concerning feature of hookworm infection is that it may induce immune suppression through its excretory/secretory products (ESPs). Hookworm ESPs appear to impair dendritic cell function, trigger secretion of immunosuppressive cytokines, induce regulatory T-cells, modulate immune cells through NO release, and induce apoptosis of effector T-cells.⁵⁴⁻⁵⁸ A persistent impact on the immune system could also explain why continued infection with hookworms and reinfection with hookworms can occur within just a few months of anthelmintic treatment.⁵⁹ It may also account for the difficulties associated with the control of hookworm and increased incidence of other infections, such as malaria in infected patients.⁶⁰ The absence of resistance to hookworms may reflect the parasite's ability to escape host immunity. Some success in developing a human hookworm vaccine suggests that an effective protective human immune response may be inducible.⁶¹⁻⁶⁴

Studies using animal models for human intestinal helminth infections (hookworm-like nematodes, and *Trichinella spiralis*), have demonstrated that tuft cells (chemosensory cells), may be responsible for initiating the development of the expulsion mechanism, in which goblet cells proliferate and, combined with specific secretory IgA antibodies, act together to limit infection in a primary exposure.⁶⁵ These investigations offer some hope for the eventual development of effective vaccines against intestinal helminths.

Clinical Disease

In general, four potential manifestations of hookworm disease (dermatitis, pneumonia, abdominal pain, and chronic iron deficiency anemia) are determined by the stage of the infection, the route of acquisition, and the degree of worm burden.¹ Initial skin penetration by L3 larvae may not result in symptoms in previously uninfected individuals. Those experiencing repeated infections may develop a pruritic papular vesicular dermatitis at the site of larval entry, known as ground itch or dew itch.

In heavily-infected individuals, there may be symptoms of pneumonia during the migratory phase of the developmental cycle of these worms. Whether this develops with any regularity is unknown, as pulmonary symptoms attributable to hookworm infection have not been observed in experimentally infected volunteers.^{33, 66} The tissue-migrating larval stages induce circulating eosinophilia one to two months after exposure.^{66, 67}

The intestinal phase can be asymptomatic, although it can result in epigastric pain and abdominal discomfort. A syndrome known as Wakana disease occurs when large numbers of larvae are ingested and is associated with nausea, vomiting, dyspnea, and eosinophilia.^{32, 68}

Severe illness resulting from hookworm infection results only when large numbers of adult worms are in the small intestine. In most endemic areas, hookworm infections “aggregate.”⁶⁹ Anywhere from two-thirds to three-fourths of the infections in a given area are sufficiently light in intensity (numbers of worms) and remain clinically silent. The clinical features of hookworm disease usually occur only in 10–30% of individuals who harbor large numbers of worms. Certain individuals might be predisposed to acquir-

ing heavy infections in some regions based on genetics or exposure to infection.^{69, 70}

The major clinical feature of hookworm disease is iron-deficiency anemia, which results from blood loss in the intestinal tract.^{1, 8, 71-74} Intestinal blood loss is proportional to the number of hookworms in the intestine. Whether or not true iron-deficiency anemia subsequently develops depends upon the predominant species of hookworm in the intestine (*A. duodenale* is associated with larger blood loss than *N. americanus*), the iron reserves of the host, and the daily intake of iron.⁷¹⁻⁷⁴ Severe anemia is associated with lassitude, palpitations, and exertional dyspnea. This can be associated with impaired growth and development in the young and may lead to angina pectoris and congestive heart failure in older individuals. The physical signs of hookworm anemia include the signs of iron deficiency, such as pale sclera, fingernail concavities (koilonychia), and cheilitis. In addition, children may manifest a yellow-green discoloration of the skin known as chlorosis due to the similarity in color to plant leaves deficient in chlorophyll.⁷⁵ Children with severe hookworm infection also show signs of protein malnutrition that result from hookworm-associated plasma protein loss, and they may develop abdominal distension, facial edema, and hair loss.

A syndrome of infantile ancylostomiasis has been described, associated with severe anemia, melena, abdominal distension, and failure to thrive.^{36, 76} Clinical and epidemiological evidence suggests these neonates ingested *A. duodenale* through breast milk.³⁶

Chronic hookworm anemia during childhood causes physical growth stunting and deficits in childhood cognition and intellectual development.^{1, 8, 10, 11} As with other soil-transmitted helminth infections, growth retardation is often reversible by administration of

antihelminthic therapy, but intellectual and cognitive deficits cannot always be reversed by therapy.⁷⁷ Chronic hookworm anemia during pregnancy can result in prematurity or low birth weight.¹¹ Many of these chronic sequelae account for the tremendous impact of hookworms on maternal and child health. The World Bank cited hookworm as a leading cause of morbidity among school-aged children in its World Development Report.⁷⁸

Diagnosis (see Clinical Appendix)

Serological tests are not available outside of research settings. Diagnosis is typically established by the microscopic identification of characteristic eggs in the stool (Figs. 19.4, C.42). Quantitative methods for determining the number of eggs per gram of feces are available. Under some circumstances, these provide an estimate of the overall worm burden. No distinction can be made among the eggs of any hookworm species based purely on their morphology. Differentiation by light microscopy depends upon examining either larvae (Fig. 19.8), or by recovering

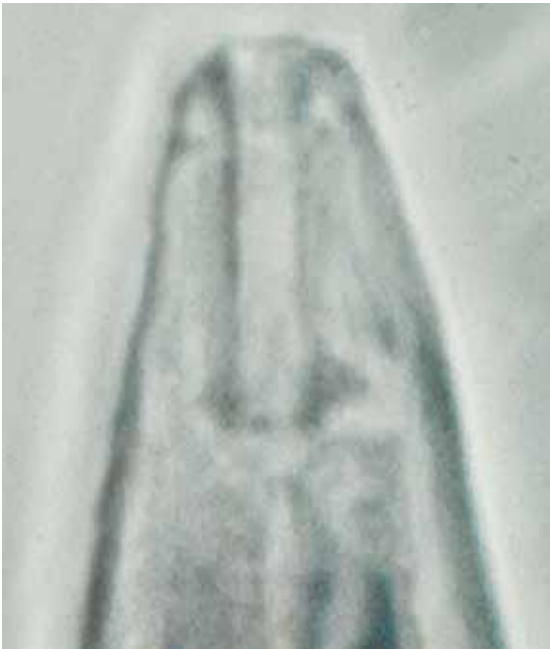


Figure 19.8. Buccal cavity of hookworm rhabditiform larva. It is longer than that of the same stage of *Strongyloides stercoralis* (see Fig. 20.6).

adult worms. PCR tests have been developed that have increased sensitivity for diagnosis and allow for species differentiation.⁷⁹ These assays are not in routine use in most parts of the world endemic for hookworm.^{80,81}

Treatment (see Clinical Appendix)

Adult hookworms are often susceptible to the two major benzimidazole anthelmintic drugs, albendazole and mebendazole, although albendazole is more effective in a single dose than a single dose of mebendazole.⁸² Failures of one dose of mebendazole or albendazole have been reported, and twice daily dosing for three days results in higher cure rates.⁸³⁻⁸⁵ Pyrantel pamoate can be given for three days as an alternate therapy, but ivermectin has poor efficacy.^{84,86}

Very young children and pregnant women are considered especially vulnerable to hookworm anemia. In Africa, hookworm was found to contribute substantially to anemia in preschool children, while in Nepal, hookworm was a significant cause of anemia in pregnancy.^{87,88} Pregnant women who received antenatal albendazole exhibited significant improvements in maternal anemia, birth weight, and infant mortality.⁸⁹ The concerns about the toxicities of the benzimidazoles need to be weighed against their potentially important benefits in pregnancy.^{90,91}

Albendazole and mebendazole work by binding to the microtubules of the parasite.⁹² Resistance to the benzimidazoles has been well-described among nematodes of veterinary importance, and occurs via mutations in the parasite tubulin alleles.⁹³ Reports on the outright failure of mebendazole to treat human hookworm infections have emerged from Mali, and the reduced efficacy of mebendazole with frequent and periodic use, have raised possible concerns that benzimidazole drug resistance may be evolving among hookworms.^{83,89} Efficacy of these drugs will

need to be monitored, as they are used in mass deworming campaigns.⁸⁹

Most children and adults with hookworm infection can be treated with benzimidazole antihelmintics alone and do not require iron supplementation. Pregnant women with severe hookworm anemia and their unborn children have been shown to benefit from simultaneous oral supplementation with iron, such as ferrous sulfate, along with antihelmintic drugs.⁸⁸

Prevention and Control

The traditional methods of hookworm control in endemic areas have included sanitary disposal of feces through the implementation of latrines, health education, drinking clean, safe water, hand washing, and cooking of food.⁹⁴ Despite decades of widespread attempts to control hookworm through these traditional methods, the global prevalence and intensity of hookworm remain the same. Among the reasons why traditional methods have failed are the ability of *A. duodenale* hookworm larvae to infect humans through ingestion, the ability of *N. americanus* larvae to penetrate all aspects of the body, including the hands and abdomen, the high rate of occupational exposure to hookworm that occurs among farm workers, and the continued reliance on untreated human feces for fertilizing crops.⁹⁵

At the World Health Assembly in 2001, member states were urged to control the global morbidity of hookworm and other STH infections through the frequent and periodic use of antihelmintic drugs. In 2012, The London Declaration on Neglected Tropical Diseases put forth a plan to control hookworm, which

involved several components, including mass drug administration.⁹⁶ There are concerns that this approach might have less of an impact on hookworm than other STH infections, such as *Trichuris* and *Ascaris*. The highest hookworm intensities in a community are often not in children, so targeting this age group is not expected to reduce hookworm transmission.⁹⁷ High rates of post-treatment hookworm reinfection occur in many communities, especially those with high transmission levels, which sometimes require thrice-yearly anthelmintic treatment.⁹⁹ A recent analysis through the Global Burden of Disease Study found that the prevalence rate of hookworm has decreased only 5% in recent decades despite comparatively greater reductions of 25% for ascariasis.⁶ The WHO NTD Roadmap targets for 2030 will need to be met with currently less than a quarter of endemic countries achieving 75% treatment coverage in preschool and school-aged children.⁹⁸

The Human Hookworm Vaccine Initiative has developed a recombinant vaccine targeting adult hookworm blood feeding by eliciting anti-enzyme antibodies that block parasite hemoglobin digestion as a complementing control strategy.⁶² This vaccine comprised of either Na-GST-1 or Na-APR-1 is being studied for its safety and immunogenicity in Gabon, Brazil and elsewhere.^{99, 100} Mathematical modeling of the vaccine indicates that it could reduce hookworm transmission, which would not be possible using pediatric anthelmintic drug deworming approaches. In addition, economic modeling indicates that a hookworm vaccine could be highly cost-effective.^{101, 102} An Na-GST-1 human hookworm vaccine has shown high levels of efficacy in a human challenge model and may be advanced for product development.¹⁰³

References

1. Hotez, P. J., et al., Hookworm infection. *The New England journal of medicine* **2004**, 351 (8), 799-807.
2. Bethony, J., et al., Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* **2006**, 367 (9521), 1521-32.
3. Ngui, R., et al., Epidemiological and genetic data supporting the transmission of *Ancylostoma ceylanicum* among human and domestic animals. *PLoS Negl Trop Dis* **2012**, 6 (2), e1522.
4. DeSilva, N. R.; Brooker, S., Soil-transmitted helminth infections: updating the global picture. *Trends Parasitol* **2003**, 19 547-51.
5. Pullan, R. L., et al., Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasit Vectors* **2014**, 7, 37.
6. Global Burden of Disease Study, C., Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **2015**, 386 (9995), 743-800.
7. IHME, Hookworm disease — Level 4 cause. https://www.healthdata.org/results/gbd_summaries/2019/hookworm-disease-level-4-cause accessed May 1, 2024 **2024**.
8. Hotez, P. J., et al., Hookworm: “the great infection of mankind”. *PLoS medicine* **2005**, 2 (3), e67.
9. Bartsch, S. M., et al., The Global Economic and Health Burden of Human Hookworm Infection. *PLoS Negl Trop Dis* **2016**, 10 (9), e0004922.
10. Brooker, S., et al., Human hookworm infection in the 21st century. *Advances in parasitology* **2004**, 58, 197-288.
11. Sakti, H., et al., Evidence for an association between hookworm infection and cognitive function in Indonesian school children. *Tropical medicine & international health : TM & IH* **1999**, 4 (5), 322-34.
12. Bundy, D. A., et al., Hookworm infection in pregnancy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1995**, 89 (5), 521-2.
13. McKenna, M. L., et al., Human Intestinal Parasite Burden and Poor Sanitation in Rural Alabama. *Am J Trop Med Hyg* **2017**, 97 (5), 1623-1628.
14. Blum, A. J.; Hotez, P. J., Global “worming”: Climate change and its projected general impact on human helminth infections. *PLoS Negl Trop Dis* **2018**, 12 (7), e0006370.
15. Kladkempetch, D., et al., *Ancylostoma ceylanicum*: The Neglected Zoonotic Parasite of Community Dogs in Thailand and Its Genetic Diversity among Asian Countries. *Animals (Basel)* **2020**, 10 (11).
16. Traub, R. J., *Ancylostoma ceylanicum*, a re-emerging but neglected parasitic zoonosis. *Int J Parasitol* **2013**, 43 (12-13), 1009-15.
17. George, S., et al., Identification of *Ancylostoma ceylanicum* in children from a tribal community in Tamil Nadu, India using a semi-nested PCR-RFLP tool. *Trans R Soc Trop Med Hyg* **2015**, 109 (4), 283-5.
18. Phosuk, I., et al., Molecular detection of *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, and *Necator americanus* in humans in northeastern and southern Thailand. *Korean J Parasitol* **2013**, 51 (6), 747-9.
19. Thompson, R. C., Neglected zoonotic helminths: *Hymenolepis nana*, *Echinococcus canadensis* and *Ancylostoma ceylanicum*. *Clin Microbiol Infect* **2015**, 21 (5), 426-32.
20. Liu, Y., et al., The zoonotic risk of *Ancylostoma ceylanicum* isolated from stray dogs and cats in Guangzhou, South China. *Biomed Res Int* **2014**, 2014, 208759.
21. Prociv, P.; Croese, J., Human eosinophilic enteritis caused by dog hookworm *Ancylostoma caninum*. *Lancet (London, England)* **1990**, 335 (8701), 1299-302.
22. Blackwell, V.; Vega-Lopez, F., Cutaneous larva migrans: clinical features and management of 44 cases presenting in the returning traveller. *The British journal of dermatology* **2001**, 145 (3), 434-7.
23. Cox, F. E., History of human parasitology. *Clin Microbiol Rev* **2002**, 15 (4), 595-612.
24. Ferreira, L. F.; Araujo, A., On hookworms in the Americas and trans-Pacific contact. *Parasitol*

- Today* **1996**, 12 (11), 454; author reply 454.
25. Dubini, A., Nuovo verme intestinale umano *Ancylostoma duodenale*, costituente un sesto genere dei nematodei proprii dell'uomo. *Ann Univ Med Milano* **1843**, 106, 5-13.
 26. Schad, G. A., et al., Arrested development in human hookworm infections: an adaptation to a seasonally unfavorable external environment. *Science (New York, N.Y.)* **1973**, 180 (4085), 502-4.
 27. Maldonado, A. E., Hookworm disease: Puerto Rico's secret killer. *P R Health Sci J* **1993**, 12 (3), 191-6.
 28. Bleakley, H., Disease and Development: Evidence from Hookworm Eradication in the American South. *Q J Econ* **2007**, 122 (1), 73-117.
 29. Stiles, C. W., A new species of hookworm (*Uncinaria americana*) parasitic in man. *Am Med* **1902**, 3, 777-778.
 30. Stoll, N. R., On endemic hookworm, where do we stand today? *Exp Parasitol* **1962**, 12, 241-51.
 31. Brooker, S., et al., Human hookworm infection in the 21st century. *Adv Parasitol* **2004**, 58, 197-288.
 32. Kojima, S., [Wakana disease]. *Ryokibetsu Shokogun Shirizu* **1999**, (24 Pt 2), 437-8.
 33. Nawalinski, T. A.; Schad, G. A., Arrested development in *Ancylostoma duodenale*: course of a self-induced infection in man. *Am J Trop Med Hyg* **1974**, 23 (5), 895-8.
 34. Hoagland, K. E.; Schad, G. A., *Necator americanus* and *Ancylostoma duodenale*: life history parameters and epidemiological implications of two sympatric hookworms of humans. *Experimental parasitology* **1978**, 44 (1), 36-49.
 35. Plamer, E. D.; J., Course of egg output over a 15-year period in a case of experimentally induced necatoriasis americanus, in the absence of hyperinfection. *The American journal of tropical medicine and hygiene* **1955**, 4 (4), 756-757.
 36. Yu, S. H., et al., Infantile hookworm disease in China. A review. *Acta tropica* **1995**, 59 (4), 265-70.
 37. Hawdon, J. M.; Hotez, P. J., Hookworm: developmental biology of the infectious process. *Current opinion in genetics & development* **1996**, 6 (5), 618-23.
 38. Zhan, B., et al., A developmentally regulated metalloprotease secreted by host-stimulated *Ancylostoma caninum* third-stage infective larvae is a member of the astacin family of proteases. *Molecular and biochemical parasitology* **2002**, 120 (2), 291-6.
 39. Asojo, O. A., et al., X-ray structure of Na-ASP-2, a pathogenesis-related-1 protein from the nematode parasite, *Necator americanus*, and a vaccine antigen for human hookworm infection. *Journal of molecular biology* **2005**, 346 (3), 801-14.
 40. Cappello, M., et al., *Ancylostoma caninum* anticoagulant peptide: a hookworm-derived inhibitor of human coagulation factor Xa. *Proceedings of the National Academy of Sciences of the United States of America* **1995**, 92 (13), 6152-6.
 41. Stanssens, P., et al., Anticoagulant repertoire of the hookworm *Ancylostoma caninum*. *Proc Natl Acad* **1996**, 93, 2149-2154.
 42. Ma, D., et al., The structure of hookworm platelet inhibitor (HPI), a CAP superfamily member from *Ancylostoma caninum*. *Acta Crystallogr F Struct Biol Commun* **2015**, 71 (Pt 6), 643-9.
 43. Del Valle, A., et al., Isolation and molecular cloning of a secreted hookworm platelet inhibitor from adult *Ancylostoma caninum*. *Mol Biochem Parasitol* **2003**, 129 (2), 167-77.
 44. Chadderdon, R. C.; Cappello, M., The hookworm platelet inhibitor: functional blockade of integrins GPIIb/IIIa (alphaIIb beta3) and GPIa/IIa (alpha2 beta1) inhibits platelet aggregation and adhesion in vitro. *J Infect Dis* **1999**, 179 (5), 1235-41.
 45. Roche, M., et al., The nature and causes of "hookworm anemia". *Am Med Hyg* **1996**, 15, 1031-1102.
 46. Don, T. A., et al., A pore-forming haemolysin from the hookworm, *Ancylostoma caninum*. *International journal for parasitology* **2004**, 34 (9), 1029-35.
 47. Williamson, A. L., et al., A multi-enzyme cascade of hemoglobin proteolysis in the intestine of blood-feeding hookworms. *The Journal of biological chemistry* **2004**, 279 (34), 35950-7.
 48. Asojo, O. A., et al., X-ray structures of Na-GST-1 and Na-GST-2 two glutathione S-transferase from the human hookworm *Necator americanus*. *BMC Struct Biol* **2007**, 7, 42.
 49. Gupta, M. C., et al., Gastrointestinal protein loss in hookworm and roundworm infections. *Am J Clin Nutr* **1974**, 27 (12), 1386-9.

50. Chu, D., et al., Molecular characterization of *Ancylostoma ceylanicum* Kunitz-type serine protease inhibitor: evidence for a role in hookworm-associated growth delay. *Infect Immun* **2004**, 72 (4), 2214-21.
51. Gaze, S., et al., Immunology of experimental and natural human hookworm infection. *Parasite Immunol* **2014**, 36 (8), 358-66.
52. Pritchard, D. I., et al., Epidemiology and immunology of *Necator americanus* infection in a community in Papua New Guinea: humoral responses to excretory-secretory and cuticular collagen antigens. *Parasitology* **1990**, 100 Pt 2, 317-26.
53. Palmer, D. R., et al., IgG4 responses to antigens of adult *Necator americanus*: potential for use in large-scale epidemiological studies. *Bulletin of the World Health Organization* **1996**, 74 (4), 381-6.
54. Segura, M., et al., Impairment of dendritic cell function by excretory-secretory products: a potential mechanism for nematode-induced immunosuppression. *Eur J Immunol* **2007**, 37 (7), 1887-904.
55. Ricci, N. D., et al., Induction of CD4(+)CD25(+)FOXP3(+) regulatory T cells during human hookworm infection modulates antigen-mediated lymphocyte proliferation. *PLoS Negl Trop Dis* **2011**, 5 (11), e1383.
56. Dondji, B., et al., Role for nitric oxide in hookworm-associated immune suppression. *Infect Immun* **2008**, 76 (6), 2560-7.
57. Chow, S. C., et al., The human hookworm pathogen *Necator americanus* induces apoptosis in T lymphocytes. *Parasite Immunol* **2000**, 22 (1), 21-9.
58. Quinnell, R. J., et al., The immunoepidemiology of human hookworm infection. *Parasite Immunol* **2004**, 26 (11-12), 443-54.
59. Albonico, M., et al., Rate of reinfection with intestinal nematodes after treatment of children with mebendazole or albendazole in a highly endemic area. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1995**, 89 (5), 538-41.
60. Nacher, M., et al., Intestinal helminth infections are associated with increased incidence of *Plasmodium falciparum* malaria in Thailand. *J Parasitol* **2002**, 88 (1), 55-8.
61. Kumar, S.; Pritchard, D. I., Skin penetration by ensheathed third-stage infective larvae of *Necator americanus*, and the host's immune response to larval antigens. *International journal for parasitology* **1992**, 22 (5), 573-9.
62. Hotez, P. J., et al., The Human Hookworm Vaccine. *Vaccine* **2013**, 31 Suppl 2, B227-32.
63. Bottazzi, M. E., The human hookworm vaccine: recent updates and prospects for success. *J Helminthol* **2015**, 89 (5), 540-4.
64. Pearson, M. S., et al., New tools for NTD vaccines: A case study of quality control assays for product development of the human hookworm vaccine Na-APR-1M74. *Hum Vaccin Immunother* **2015**, 11 (5), 1251-7.
65. Howitt, M. R., et al., Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* **2016**, 351 (6279), 1329-33.
66. Maxwell, C., et al., The clinical and immunologic responses of normal human volunteers to low dose hookworm (*Necator americanus*) infection. *Am J Trop Med Hyg* **1987**, 37 (1), 126-34.
67. White, C. J., et al., Changes in the structural and functional properties of human eosinophils during experimental hookworm infection. *J Infect Dis* **1986**, 154 (5), 778-83.
68. Yoshida, Y., et al., Experimental studies on the infection mode of *Ancylostoma duodenale* and *Necator americanus* to the definitive host. *Japanese* **1958**, 7, 102-112.
69. Schad, G. A.; Anderson, R. M., Predisposition to hookworm infection in humans. *Science (New York, N.Y.)* **1985**, 228 (4707), 1537-40.
70. Quinnell, R. J., et al., Predisposition to hookworm infection in Papua New Guinea. *Trans R Soc Trop Med Hyg* **2001**, 95 (2), 139-42.
71. Albonico, M., et al., Epidemiological evidence for a differential effect of hookworm species, *Ancylostoma duodenale* or *Necator americanus*, on iron status of children. *International journal of epidemiology* **1998**, 27 (3), 530-7.
72. Stoltzfus, R. J., et al., Hookworm control as a strategy to prevent iron deficiency. *Nutrition reviews* **1997**, 55 (6), 223-32.

73. Stoltzfus, R. J., et al., Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: the importance of hookworms. *The American journal of clinical nutrition* **1997**, 65 (1), 153-9.
74. Stoltzfus, R. J., et al., Hemoquant determination of hookworm-related blood loss and its role in iron deficiency in African children. *The American journal of tropical medicine and hygiene* **1996**, 55 (4), 399-404.
75. Crosby, W. H., Whatever became of chlorosis? *JAMA* **1987**, 257 (20), 2799-800.
76. Hotez, P. J., Hookworm disease in children. *The Pediatric infectious disease journal* **1989**, 8 (8), 516-20.
77. Stephenson, L. S., et al., Treatment with a single dose of albendazole improves growth of Kenyan schoolchildren with hookworm, *Trichuris trichiura*, and *Ascaris lumbricoides* infections. *The American journal of tropical medicine and hygiene* **1989**, 41 (1), 78-87.
78. Bank, T. W., World Development Report 1993: Investing in Health. Oxford University Press, Inc, New York: 1993.
79. Mejia, R., et al., A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited at-risk populations. *Am J Trop Med Hyg* **2013**, 88 (6), 1041-7.
80. van Mens, S. P., et al., Comparison of real-time PCR and Kato smear microscopy for the detection of hookworm infections in three consecutive faecal samples from schoolchildren in Ghana. *Trans R Soc Trop Med Hyg* **2013**, 107 (4), 269-71.
81. Phuphisut, O., et al., Triplex polymerase chain reaction assay for detection of major soil-transmitted helminths, *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, in fecal samples. *Southeast Asian J Trop Med Public Health* **2014**, 45 (2), 267-75.
82. Albonico, M., et al., Control strategies for human intestinal nematode infections. *Advances in parasitology* **1999**, 42, 277-341.
83. DeClercq, D., et al., Failure of mebendazole in treatment of human hookworm infections in the southern region of Mali. *Am Med Hyg* **1997**, 57 25-30.
84. Steinmann, P., et al., Efficacy of single-dose and triple-dose albendazole and mebendazole against soil-transmitted helminths and *Taenia* spp.: a randomized controlled trial. *PloS one* **2011**, 6 (9), e25003.
85. Soukhathammavong, P. A., et al., Low efficacy of single-dose albendazole and mebendazole against hookworm and effect on concomitant helminth infection in Lao PDR. *PLoS Negl Trop Dis* **2012**, 6 (1), e1417.
86. Keiser, J.; Utzinger, J., Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *JAMA* **2008**, 299 (16), 1937-48.
87. Brooker, S., et al., The epidemiology of hookworm infection and its contribution to anaemia among pre-school children on the Kenyan coast. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1999**, 93 (3), 240-6.
88. Christian, P., et al., Antenatal anthelmintic treatment, birthweight, and infant survival in rural Nepal. *Lancet (London, England)* **2004**, 364 (9438), 981-3.
89. Albonico, M., et al., Monitoring drug efficacy and early detection of drug resistance in human soil-transmitted nematodes: a pressing public health agenda for helminth control. *International journal for parasitology* **2004**, 34 (11), 1205-10.
90. Ndibazza, J., et al., Effects of deworming during pregnancy on maternal and perinatal outcomes in Entebbe, Uganda: a randomized controlled trial. *Clin Infect Dis* **2010**, 50 (4), 531-40.
91. Torp-Pedersen, A., et al., Exposure to mebendazole and pyrvinium during pregnancy: a Danish nationwide cohort study. *Infect Dis Obstet Gynecol* **2012**, 2012, 769851.
92. Fennell, B., et al., Microtubules as antiparasitic drug targets. *Expert Opin Drug Discov* **2008**, 3 (5), 501-18.
93. Von Samson-Himmelstjerna, G., et al., Single nucleotide polymorphism (SNP) markers for benzimidazole resistance in veterinary nematodes. *Parasitology* **2007**, 134 (Pt 8), 1077-86.
94. Zheng, Q., et al., The control of hookworm infection in China. *Parasit Vectors* **2009**, 2 (1), 44.
95. Humphries, D. L., et al., The use of human faeces for fertilizer is associated with increased intensity of hookworm infection in Vietnamese women. *Transactions of the Royal Society of*

- Tropical Medicine and Hygiene* **1997**, 91 (5), 518-20.
96. Anderson, R., et al., The coverage and frequency of mass drug administration required to eliminate persistent transmission of soil-transmitted helminths. *Philos Trans R Soc Lond B Biol Sci* **2014**, 369 (1645), 20130435.
 97. Chan, M. S., et al., Transmission patterns and the epidemiology of hookworm infection. *International journal of epidemiology* **1997**, 26 (6), 1392-400.
 98. Organization, W. H., Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva. Licence: CC BY-NC-SA 3.0 IGO **2020**.
 99. Adegnik, A. A., et al., Safety and immunogenicity of co-administered hookworm vaccine candidates Na-GST-1 and Na-APR-1 in Gabonese adults: a randomised, controlled, double-blind, phase 1 dose-escalation trial. *Lancet Infect Dis* **2021**, 21 (2), 275-285.
 100. Zinsou, J. F., et al., Safety and immunogenicity of the co-administered Na-APR-1 and Na-GST-1 hookworm vaccines in school-aged children in Gabon: a randomised, controlled, observer-blind, phase 1, dose-escalation trial. *Lancet Infect Dis* **2024**.
 101. Bartsch, S. M., et al., Modeling the economic and epidemiologic impact of hookworm vaccine and mass drug administration (MDA) in Brazil, a high transmission setting. *Vaccine* **2016**, 34 (19), 2197-206.
 102. Lee, B. Y., et al., The potential economic value of a hookworm vaccine. *Vaccine* **2011**, 29 (6), 1201-10.
 103. Puchner, K. P., et al., Vaccine value profile for Hookworm. *Vaccine* **2023**.



Giovanni Battista Grassi, M.D. (1854–1925)

Grassi was a parasitologist “for all seasons”, working diligently on a plethora of biological systems, including: invertebrate development (termites and honey bees), parasite life cycles (*Ascaris lumbricoides*, *Strongyloides stercoralis*, *Ancylostoma duodenale*, filaria of various species, *Hymenolepis nana*, *Plasmodium vivax* (co-discoverer with R. Feletti), vectors of human malaria, and plant pathogens affecting wine grapes.

Grassi was a thorough, observant investigator, and made significant contributions to the advancement of knowledge in each of these fields. His worldwide fame arose from his research, in collaboration with Amico Bignami, Guiseppe Bastienelli, and Antonio Dionisi, he identified the female *Anopheles* mosquito as the vector of human malaria. Grassi also linked the species of plasmodia with its malarial fever pattern.

20. *Strongyloides stercoralis*

(Bavay 1876)

Pronunciation: \strän-jə-'loi-,dēz\

Introduction

Strongyloides stercoralis (\STRON-ji-LOI-deez\)) is a parasitic nematode with a worldwide distribution. It is prevalent throughout tropical, subtropical, and temperate climates. In Sub-Saharan Africa, South East Asia (e.g., Cambodia), parts of the Caribbean, and South America, some prevalence rates are higher than 20%.¹ In North America, it occurs frequently among immigrants, in parts of Appalachia, and in patients at long-term healthcare facilities.^{2,4} Because of the difficulty in establishing a definitive diagnosis and because the parasite may cause long-lasting asymptomatic infections, the true global prevalence of human strongyloidiasis is unknown. Estimates indicate there may be as many as 100 million cases/year.^{2,5}



Figure 20.1. Free-living adult of *Strongyloides stercoralis*. This stage occurs in soil only. 600 μ m.

Dogs and non-human primates are important reservoirs.⁶ There have been numerous outbreaks of strongyloidiasis among dog handlers.⁷ *S. stercoralis* can also reproduce as a free-living nematode in soil, and the L3 (filariform) larva retains its ability to infect mammalian hosts. This qualifies *S. stercoralis* as one of the most environmentally adaptable nematode infections of humans.

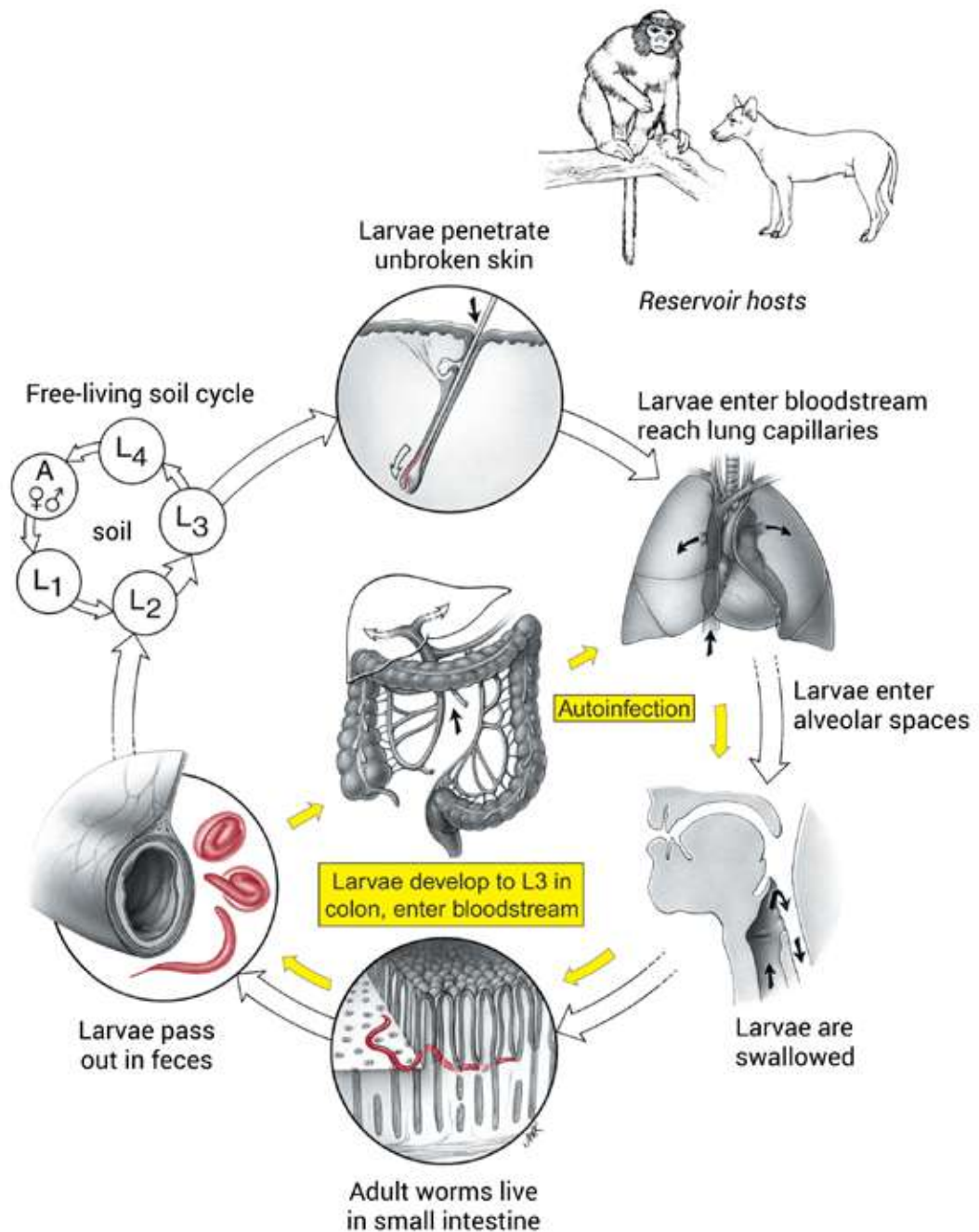
A second form of human strongyloidiasis, caused by *S. fuelleborni*, has been described in infants living in Papua New Guinea, Sub-Saharan Africa, and South America.⁸⁻¹⁰ Children with this infection can develop a special clinical syndrome called swollen belly syndrome.¹¹ This condition is associated with a high rate of mortality. In some rural villages, the prevalence of *S. fuelleborni* may reach nearly 100% during the early years of life; then, it declines in older children and adults.¹²

Historical Information

Clinical infection with *S. stercoralis* was first described by Arthur Bavay and Louis Normand in 1876, while they were working together in Toulon, France.^{13,14} Their patients were French army personnel, newly arrived from Cochin, Indochina (modern-day southern Vietnam); thus, the term for strongyloid enteritis, “Cochin China diarrhea.” Bavay recovered numerous larvae of a species of nematode that had not been previously described from the stool of these patients and named it *Anguillula stercoralis*.¹⁵

Max Askanazy described the pathology of strongyloidiasis, and Friedrich Fuelleborn reported the life cycle.^{16,17} Fuelleborn conducted animal experiments and learned that infective larvae penetrate unbroken skin. In 1928, Masao Nishigoi described autoinfection with *S. stercoralis*, and also showed that infected dogs made constipated with morphine and bismuth subnitrate passed infec-

Strongyloides stercoralis



tive L3 larvae, rather than non-infective L2 (second-stage rhabditiform) larvae.¹⁸ In addition, he noted that the number of larvae passed by these animals increased so long as constipation was maintained. These studies preceded the clinical description of autoinfection, now known to also occur in humans.

Life Cycle

S. stercoralis is a soil-transmitted helminth (STH) that exists both as a free-living (Fig. 20.1) and as a parasitic nematode (Fig. 20.2a). The parasitic adult female is approximately 2 mm by 35 μ m, and lives embedded within a row of columnar epithelial cells in the small intestine (Fig. 20.2b), usually in the region of the duodenum and proximal jejunum. Adult worms may live for up to 5 years. Reproduction is by parthenogenesis during this portion of the life cycle, releasing fertilized, unembryonated eggs into the lamina propria.¹⁹



Figure 20.2a. Parasitic female *S. stercoralis*. 2.5 mm x 35 μ m. Courtesy L. Ash and T. Orihel.

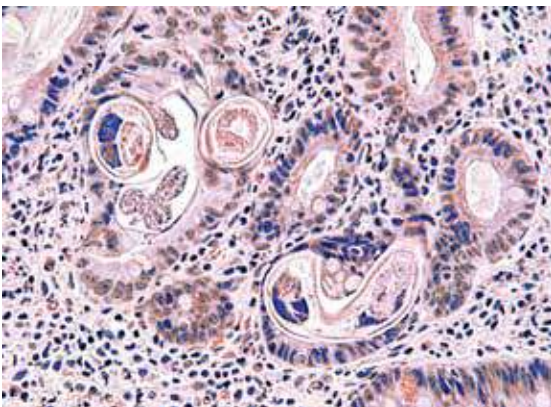


Figure 20.2b. Small intestine with numerous sections of a parasitic female *S. stercoralis*.

The eggs embryonate rapidly and hatch into L1 (rhabditiform) larvae, which emerge into the lumen of the small intestine. Larvae proceed to the colon, where they molt once, becoming L2 (second-stage rhabditiform) larvae that can then be deposited in soil with feces. Alternatively, they may molt into L3 (filariform) larvae while still within the colon's lumen, burrow into the mucosa, and enter the circulation directly or through the perianal skin. This process is known as autoinfection.²⁰

Free-living phase L2 larvae require warm, moist, sandy, or loamy soil for the next developmental phase of the cycle to take place. Under optimal environmental conditions, they develop into free-living adult worms in the appropriate soil. This occurs by several successive molts. In contrast to the parthenogenic portion of the life cycle in the mammalian host, adult worms of both sexes are found during the free-living phase in soil.¹⁹ Free-living males and females die after a full cycle. After mating, the female produces embryonated eggs that hatch as L1 larvae and develop into L2 larvae that then molt to become infectious L3 larvae.

The L3 larva infects humans and other susceptible hosts. When conditions become unfavorable for the continuation of the free-



Figure 20.3. Rhabditiform larva of *S. stercoralis*. 580 μ m x 15 μ m.

living phase (e.g., lack of nutrition, low moisture), more L2 larvae transform into the infective L3 stage that can remain in the soil for several days.²¹ When the L3 larvae encounter a suitable host, they penetrate the skin (Fig. 20.4) and begin the parasitic phase of the infection. L3 larvae can also “swim” in aquatic environments, giving them a greater range to find a host than hookworm L3 larvae, which cannot do so. The free-living life cycle can only occur for one generation with *S. stercoralis*. After that, the nematode must enter a host for further reproductive cycles to occur.

Parasitic Phase (Homogonic Life Cycle)

The L3 larva enters the host through the skin, facilitated by a protease secreted by the parasite.²² Upon entry into the host, the immature worm probably enters a venule and/or lymphatic vessel before being carried through the afferent circulation through the right heart, pulmonary artery, and pulmonary capillaries.

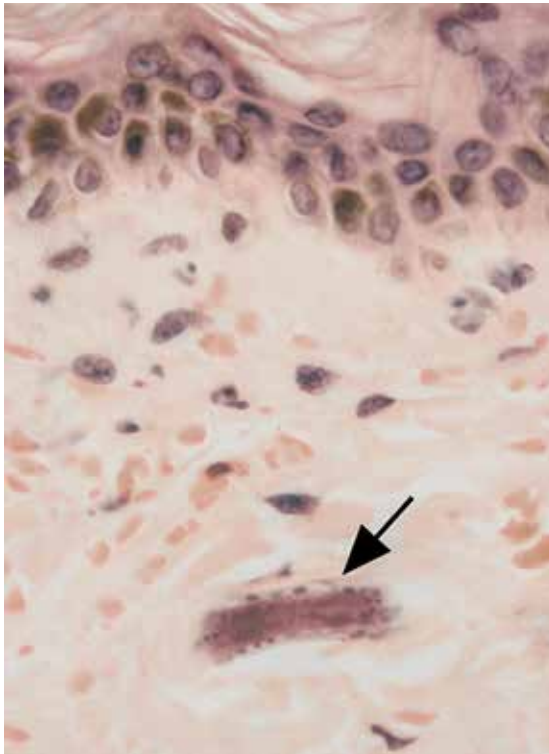


Figure 20.4. Filariform larva (arrow) of *S. stercoralis* in cutaneous layer of skin.

The larva ruptures into the alveolar space, actively crawls up the respiratory tree, passes through the trachea into the pharynx, crosses the epiglottis, and is swallowed, *a la* Ascaris and hookworm. *S. stercoralis* may not always migrate through the lungs to reach the intestinal tract.²³ The larva undergoes a final molt in the small intestine, developing into the parasitic parthenogenic female. Egg production begins within 25–30 days after the initial infection.

Autoinfection, Hyperinfection, and Disseminated Infection

In some patients, L2 larvae develop within the colon to the infective L3 stage.²⁴ The infective larvae may reenter the circulation before they migrate through the lungs and are swallowed. This process is called autoinfection and allows the parasite to remain inside the same host for many years. Low levels of autoinfection are thought to be common and may occur during a primary infection.²⁵ In debilitated, malnourished, or immunocompromised patients, autoinfection can amplify, leading to hyperinfection characterized by a large increase in the worm burden. *S. stercoralis* is one of the few parasitic nematodes infecting humans that can increase its numbers within the same individual (the other being *Capillaria philippinensis*; Fig. 26.1). Hyperinfection can also lead to disseminated infection, characterized by the presence of various stages of larvae at ectopic sites, including the CNS.

Vertical Transmission

The mode of transmission of *S. fuelleborni* infection leading to swollen belly syndrome is unknown. The high incidence of this parasite in infants has led to the speculation that it is transmitted through mothers' milk. In support of this notion, vertical transmission of *S. stercoralis* has been demonstrated in dogs. It may also occur in humans, and the larvae of *S. fuelleborni* have been demonstrated in mammary secretions.²⁶

Cellular and Molecular Pathogenesis

Parasitic females induce essentially no damage to the small intestine's mucosa, but elicit local inflammation. In some experimental studies, T-cell function appears to be necessary for the development of resistance to *Strongyloides* infection and may reflect the same immune cascade elicited by tuft cells in the small intestine.^{21, 27} Impairment of T-cell function has been proposed as the basis by which subsets of infected individuals fail to regulate the number of worms in their small intestines, developing *Strongyloides* hyperinfection and disseminated infection. This is evident in patients with immunosuppression due to malignancy, human T-cell lymphotropic virus type 1 (HTLV-1) infection, alcoholism, malnutrition, corticosteroids, and cytotoxic medications.^{20, 28-31} Despite a few reports of disseminated *Strongyloides* in patients with HIV/AIDS, the significantly higher-than-expected incidence of hyperinfection and disseminated *Strongyloides* infections has not been seen in HIV-1-infected patients.³²⁻³⁴ Speculation focuses on the fact that hyperinfection occurs in response to elevated steroid levels in patients at risk for these conditions.^{32, 33} The steroid effect may be mediated by their ability to suppress eosinophilia, interfere with lymphocyte activation, or have a direct impact on the parasitic larvae that accelerates their maturation into invasive L3 larvae while still within the colon of the infected patient.²¹

The association between HTLV-1 infection and strongyloidiasis was observed on the islands of Okinawa and Jamaica and is now broadly appreciated.³⁵⁻³⁷ The basis for this association is not fully understood, although it is suggested that patients with the disease may have selective deficits in parasite-specific antibodies. The mechanism probably involves IgE and induced T-cell tolerance

for *Strongyloides* due to HTLV-1 tropism for regulatory T-cells.^{37, 38} Severe strongyloidiasis has also been described in an IgA-deficient patient, but a causal connection between IgA deficiency and *Strongyloides* is unclear.³⁷

In hyperinfection or disseminated infection cases, penetrating L3 larvae often carry enteric microorganisms in their gut tract that they fed upon during their life as L2 larvae. As they develop further, they regurgitate these microbes throughout the host's tissues, often leading to local infection/bacteremia, followed by general sepsis.³⁹ Such clinical sequelae are frequently fatal.⁴⁰

Clinical Disease

Following infection of immunocompetent individuals, there may be no prominent symptoms or a watery, mucous diarrhea, the degree of which varies with the intensity of the infection. Most infected patients display no symptoms following infection; peripheral eosinophilia may be the only evidence of acute infection.^{41, 42} Some individuals may report skin reactions, commonly called ground itch, due to skin penetration by the infecting L3 larvae.⁴³ The rash of ground itch is characteristically on the feet. In approximately 25% of symptomatic patients, states of alternating diarrhea and constipation, abdominal discomfort, vomiting, and epigastric pain that worsens with eating have been reported.^{41, 44} Although these symptoms typically last about six weeks, some individuals may have persistent symptoms for years.⁴⁵ Children with *S. stercoralis* may develop a syndrome characterized by anorexia, cachexia, chronic diarrhea, fat and protein malabsorption, and abdominal distension. They can have impaired growth, which is reversible after specific antihelminthic chemotherapy.⁴⁶ Infants with swollen belly syndrome from *S. fuelleborni* may present acutely but usually without fever and diarrhea.^{8, 9} Instead,

these children develop abdominal ascites due to protein loss, which can accumulate to the point of causing respiratory embarrassment.

During the migratory phase of the infection, symptoms may resemble those described for ascariasis and hookworm disease (e.g., pneumonitis), although in the absence of disseminated infection, pulmonary symptoms are not usually prominent. More commonly, pulmonary strongyloidiasis is characterized by asymptomatic circulating eosinophilia.⁴⁷ Migration of larvae through the skin gives rise to a serpiginous, creeping urticarial eruption, a condition known as *larva currens*.⁴⁸ Larvae have been observed to migrate through the skin as fast as 5–15 cm per hour, leaving intense red pruritic streaks on the abdomen or lateral thighs.⁴⁴ Strongyloidiasis may also present with a petechial purpuric rash (periumbilical parasitic thumbprint purpura) that is commonly present on the anterior abdomen or lateral thighs.^{44, 49, 50} There are two distinct rashes associated with disseminated strongyloidiasis: *larva currens* and periumbilical parasitic thumbprint purpura.

Hyperinfection and Disseminated Infection

Clinical symptoms are exaggerated if hyperinfection is superimposed on an already chronic infection.⁵¹ Disseminated infection may alternatively occur outside of the context of any clinically apparent chronic infection. In this context an individual may have a latent *Strongyloides* infection and, in the context of immunosuppression, may develop disseminated disease. Hyperinfection has been reported in many immunosuppressive conditions, with many immunosuppressive agents and anti-inflammatories. Glucocorticoids are widely used and are specifically associated with this syndrome. The ability of glucocorticoids to induce this syndrome is probably multifactorial. This may be due to their suppression of eosinophils, inhibition of

lymphocyte activation, and possibly the ability to accelerate the transformation of L2 second-stage rhabditiform larvae to invasive L3 filariform larvae, perhaps through their structural similarity to ecdysteroid hormones.²⁰

²¹ Corticosteroids are hypothesized to act as molting signals for the transformation of larvae in the invasive filariform larvae.²¹

Massive invasion by *Strongyloides* larvae due to hyperinfection has an impressive presentation as acute enteritis, with severe diarrhea and ulcerating disease of the small and large intestines. During disseminated infection, the L3 larvae may enter the CNS and result in gram-negative meningitis, and in some cases, secondary abscesses, both caused by regurgitated enteric microbes.⁵²⁻⁵⁵ Pulmonary invasion is also exaggerated during disseminated infection and can lead to a clinical presentation of pneumonia, pulmonary embolism, intrapulmonary hemorrhage, or acute respiratory failure.⁵⁶

Diagnosis (see Clinical Appendix)

Strongyloides infection should be considered in patients with unexplained gastrointestinal (GI) symptoms, with or without eosinophilia, and an appropriate exposure history.⁵⁷ Identification of the larvae in stool samples is the definitive method of diagnosis. (Figs. 20.3, 20.6). Because few organisms are intermit-

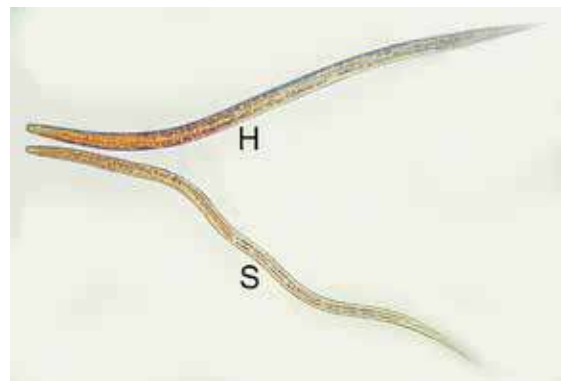


Figure 20.5. Filariform (L3) larvae. H = Hookworm and S = *Strongyloides*. Courtesy L. Ash and T. Orihel.



Figure 20.6. Buccal cavity (arrow) of the rhabditiform larva of *S. stercoralis*. Compare with Figure 19.8.

tently released into the stool, the sensitivity of a standard single stool examination is less than 50%, and even as low as 30% by some estimates.^{2,58,59} As few as 50 larvae are released daily by each adult female worm. Compare this reproductive output with *Ascaris*, which produces more than 200,000 eggs daily. It is highly recommended that a large quantity of stool (e.g., multiple samples on multiple days) be made available, and all of it should be processed by a sedimentation method to concentrate the larvae, greatly improving the chances of seeing the organisms on microscopic examination. Even when sedimentation techniques are used, low-grade infections are usually missed, and a rigorous search with multiple stool examinations must be carried out before a patient can be declared free of the infection. *S. stercoralis* larvae can be differentiated from those of hookworms (Fig. 20.5). The rhabditiform larva of *S. stercoralis* has a short buccal cavity (Fig. 20.6) and a notched tail (Fig. 20.7). Laboratories that are comfortable with handling the organism can enhance the sensitivity of their stool examinations by plating out a fecal pat on an agar plate and detecting the tracks of bacteria dragged along by migrating larvae. Alternatively, some laboratories can amplify the number of *Strongyloides* larvae by mixing the stool with bone charcoal and incubating

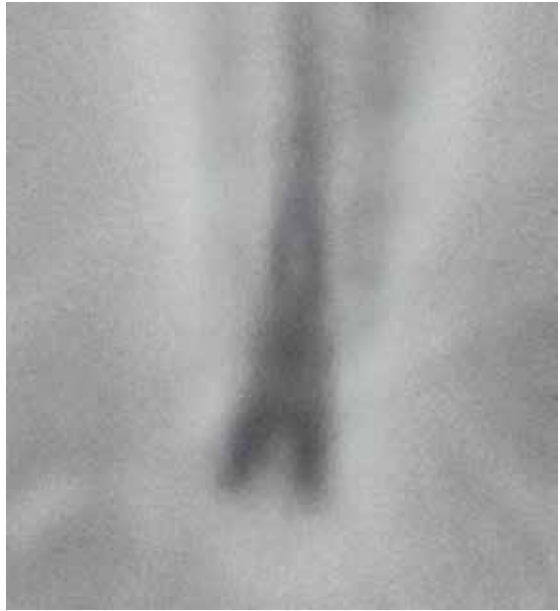


Figure 20.7. Notched tail of filariform larva of *S. stercoralis*. Hookworm L3 larva has a pointed tail.

the preparation under conditions that favor the induction of the heterogonic life cycle (coproculture).^{60,61}

In patients with hyperinfection or disseminated infection, the yield of *Strongyloides* larvae detection may be increased by recovering them from duodenal fluid.⁵⁹ Typically, this is done through flexible endoscopy; intestinal biopsy is also a useful adjunct (Fig. 20.2b). During disseminated infections, it is possible to identify the parasite from sputum and bronchoalveolar lavage fluid.²⁰ Several serological tests, including Bordier ELISA for IgG, are available, but their sensitivities and specificities can vary significantly.⁶²⁻⁶⁴ Despite their limitations, serologic testing is currently the diagnostic method of choice.⁶⁵ Immunological approaches for detecting parasite-specific antibodies in serum by ELISA can achieve a diagnostic sensitivity of 100 percent but have a lower specificity primarily because of cross-reactivity with other helminths. PCR assays are under development, including multiplex quantitative PCR assays to simultaneously diagnosis parasite co-infections.⁶⁶

Treatment (see Clinical Appendix)

While thiabendazole was administered in the past for uncomplicated infection, ivermectin has fewer side effects and has replaced it as the drug of choice.⁶⁷⁻⁷¹ Albendazole is an alternative drug but is less effective than ivermectin.⁷²

For patients with hyperinfection and disseminated disease the ideal treatment is unknown, but usually, ivermectin is extended to between 5 days and several weeks, and often albendazole is added.^{67, 68, 73-75} Treatment efficacy can be monitored based on patient response and repeated stool examinations.⁷⁶ Ivermectin treatment significantly reduces the burden of parasites but does not appear to rid the host of parasites completely, the majority of ivermectin-treated patients with no re-infection risk will continue to have parasite DNA and larvae detectable in their stools for years after treatment.⁷⁷ In some cases of severe infection, veterinary parenteral preparations of ivermectin have been used successfully in the treatment of disseminated strongyloidiasis.^{78, 79} Intensive supportive therapy, including antimicrobial agents, is often required in patients with hyperinfection from *S. stercoralis* and parenteral nutrition to compensate for extensive protein and lipid losses. *S. fuelleborni* infection is treated successfully with thiabendazole.⁸⁰

Prevention and Control

Disease risk may be greatly reduced by wearing shoes in endemic areas.⁸¹ Reservoir hosts include dogs and primates, most notably chimpanzees. As mentioned, a

small outbreak of human strongyloidiasis that originated in dogs has been described.⁷ Animal care personnel at research institutions are at high risk of acquiring strongyloidiasis. Newly arrived primates intended for use in investigative research protocols are first kept in isolation and routinely treated with ivermectin to prevent the spread of this nematode parasite. Other even more serious pathogens have been introduced to this group of employees in past years, such as Marburg virus and Ebola virus. Quarantine is the best way to ensure these serious pathogens do not cause in-house epidemics, such as the one that occurred at Hoechst in Marburg, Germany in 1967.⁸²

Custodial institutions can be a foci of infection, and consequently screening and treating of patients can prevent spread.⁸³ Efforts to diagnose and screen individuals who harbor *S. stercoralis* are sometimes carried out among patients who are candidates for immunosuppressive therapy.⁵ Treatment of pregnant women or those infected and of childbearing age would reduce transmammary transmission of *S. fuelleborni*, although many of the available antihelminthic medications are relatively contraindicated during pregnancy. Calls for addition of strongyloides as an additional helminth in a newly designed global deworming program have been made⁸⁴ and now the WHO NTD roadmap includes *S. stercoralis* in the group of soil transmitted helminths with a plan for elimination. Specifically, the roadmap calls for better diagnostic testing to define the global burden of disease and the addition of ivermectin to de-worming programs when strongyloides or trichuris are endemic.⁸⁵

References

- Hatter, A. D., et al., Case report of individual with cutaneous immunodeficiency and novel 1p36 duplication. *Appl Clin Genet* **2016**, 9, 1-4.
- Genta, R. M., et al., Strongyloidiasis in US veterans of the Vietnam and other wars. *JAMA* **1987**, 258 (1), 49-52.
- Russell, E. S., et al., Prevalence of *Strongyloides stercoralis* antibodies among a rural Appalachian population--Kentucky, 2013. *Am J Trop Med Hyg* **2014**, 91 (5), 1000-1.
- Centers for Disease, C.; Prevention, Notes from the field: *strongyloides* infection among patients at a long-term care facility--Florida, 2010-2012. *MMWR Morb Mortal Wkly Rep* **2013**, 62 (42), 844.
- Genta, R. M., Global prevalence of strongyloidiasis: critical review with epidemiologic insights into the prevention of disseminated disease. *Reviews of infectious diseases* **1989**, 11 (5), 755-67.
- Goncalves, A. L., et al., Evaluation of strongyloidiasis in kennel dogs and keepers by parasitological and serological assays. *Vet Parasitol* **2007**, 147 (1-2), 132-9.
- Georgi, J. R.; Sprinkle, C. L., A case of human strongyloidosis apparently contracted from asymptomatic colony dogs. *Am J Trop Med Hyg* **1974**, 23 (5), 899-901.
- Ashford, R. W., et al., *Strongyloides* infection associated with acute infantile disease in Papua New Guinea. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1978**, 72 (5), 554.
- Barnish, G.; Ashford, R. W., *Strongyloides cf. fuelleborni* and hookworm in Papua New Guinea: patterns of infection within the community. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1989**, 83 (5), 684-8.
- Zerpa Larrauri, R., et al., [Life cycle of *Strongyloides fuelleborni*: images and videos]. *Rev Peru Med Exp Salud Publica* **2014**, 31 (2), 390-2.
- Vince, J. D., et al., *Strongyloides* species infestation in young infants of papu, New Guinea: association with generalized oedema. *P N G Med J* **1979**, 22 (2), 120-7.
- Ashford, R. W., et al., *Strongyloides* infection in a mid-mountain Papua New Guinea community: results of an epidemiological survey. *P N G Med J* **1979**, 22 (2), 128-35.
- Bavay, A.; R., C., Sur l'anguillule stercorale. *Sci Paris* **1876**, 83, 694-696.
- Normand, L. A.; R., C., Sur la maladie dite diarrhie de Cochinchine. *Sean Acad Sci* **1876**, 83 316-318.
- Darling, S. T., *Strongyloides* Infections in Man and Animals in the Isthmian Canal Zone. *J Exp Med* **1911**, 14 (1), 1-24.
- Askanazy, M., Uber Art und Zweck der Invasion der Anguillula intestinalis in die Darmwand. *Zbl Bakt* **1900**, 27 569-578.
- Fuelleborn, F., Untersuchungen uber den Infektionsweg bei *Strongyloides* und *Ankylostomum* und die Biologie diesen Parasiten. *Arch Schiffs Trop* **1914**, 18, 26-80.
- Nishigoi, M., On various factors influencing the development of *Strongyloides stercoralis* and autoinfection. *Taiwan Sgakkai Zassi* **1928**, 27, 1-56.
- Streit, A., Reproduction in *Strongyloides* (Nematoda): a life between sex and parthenogenesis. *Parasitology* **2008**, 135 (3), 285-94.
- Keiser, P. B.; Nutman, T. B., *Strongyloides stercoralis* in the Immunocompromised Population. *Clin Microbiol Rev* **2004**, 17 (1), 208-17.
- Genta, R. M., Dysregulation of strongyloidiasis: a new hypothesis. *Clinical microbiology reviews* **1992**, 5 (4), 345-55.
- McKerrow, J. H.; Brindley, P., *Strongyloides stercoralis*: identification of a protease whose inhibition prevents larval skin invasion. *Exp Parasitol* **1990**, 70, 134-143.
- Schad, G. A., et al., *Strongyloides stercoralis*: is there a canonical migratory route through the host? *The Journal of parasitology* **1989**, 75 (5), 740-9.
- Shekamer, J. H., et al., Persistent strongyloidiasis in an immunodeficient patient. *Am Med Hyg* **1982**, 31 746-751.
- Schad, G. A., et al., *Strongyloides stercoralis*: an initial autoinfective burst amplifies primary infection. *The American journal of tropical medicine and hygiene* **1993**, 48 (5), 716-25.
- Brown, R. C.; Girardeau, H. F., Transmammary passage of *Strongyloides* sp. larvae in the human

- host. *The American journal of tropical medicine and hygiene* **1977**, 26 (2), 215-9.
27. Howitt, M. R., et al., Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* **2016**, 351 (6279), 1329-33.
28. Safdar, A., et al., Strongyloidiasis in patients at a comprehensive cancer center in the United States. *Cancer* **2004**, 100 (7), 1531-6.
29. Carvalho, E. M.; Da Fonseca Porto, A., Epidemiological and clinical interaction between HTLV-1 and *Strongyloides stercoralis*. *Parasite Immunol* **2004**, 26 (11-12), 487-97.
30. Marques, C. C., et al., Alcoholism and *Strongyloides stercoralis*: daily ethanol ingestion has a positive correlation with the frequency of *Strongyloides* larvae in the stools. *PLoS Negl Trop Dis* **2010**, 4 (6), e717.
31. Buonfrate, D., et al., Severe strongyloidiasis: a systematic review of case reports. *BMC Infect Dis* **2013**, 13, 78.
32. Lucas, S. B., Missing infections in AIDS. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1990**, 84 Suppl 1, 34-8.
33. Robinson, R. D., Parasitic infections associated with HIV/AIDS in the Caribbean. *Bulletin of the Pan American Health Organization* **1995**, 29 (2), 129-37.
34. Viney, M. E., et al., Why does HIV infection not lead to disseminated strongyloidiasis? *J Infect Dis* **2004**, 190 (12), 2175-80.
35. Dixon, A. C., et al., Strongyloidiasis associated with human T-cell lymphotropic virus type I infection in a nonendemic area. *The Western journal of medicine* **1989**, 151 (4), 410-3.
36. Arakaki, T., et al., Epidemiological aspects of *Strongyloides stercoralis* infection in Okinawa, Japan. *The Journal of tropical medicine and hygiene* **1992**, 95 (3), 210-3.
37. Neva, F. A., et al., Antibodies to *Strongyloides stercoralis* in healthy Jamaican carriers of HTLV-1. **1989**, 320, 252-3.
38. Montes, M., et al., Regulatory T cell expansion in HTLV-1 and strongyloidiasis co-infection is associated with reduced IL-5 responses to *Strongyloides stercoralis* antigen. *PLoS Negl Trop Dis* **2009**, 3 (6), e456.
39. Gorman, S. R.; Craven, D. E., Images in clinical medicine. *Strongyloides stercoralis* hyperinfection. *N Engl J Med* **2008**, 359 (11), e12.
40. Geri, G., et al., *Strongyloides stercoralis* hyperinfection syndrome: a case series and a review of the literature. *Infection* **2015**, 43 (6), 691-8.
41. Valerio, L., et al., *Strongyloides stercoralis*, the hidden worm. Epidemiological and clinical characteristics of 70 cases diagnosed in the North Metropolitan Area of Barcelona, Spain, 2003-2012. *Trans R Soc Trop Med Hyg* **2013**, 107 (8), 465-70.
42. Gonzalez, A., et al., Clinical and epidemiological features of 33 imported *Strongyloides stercoralis* infections. *Trans R Soc Trop Med Hyg* **2010**, 104 (9), 613-6.
43. Bailey, M. S., et al., Helminth infections in British troops following an operation in Sierra Leone. *Trans R Soc Trop Med Hyg* **2006**, 100 (9), 842-6.
44. Greaves, D., et al., *Strongyloides stercoralis* infection. *Bmj* **2013**, 347, f4610.
45. Vadlamudi, R. S., et al., Intestinal strongyloidiasis and hyperinfection syndrome. *Clin Mol Allergy* **2006**, 4, 8.
46. Leung, V. K., et al., Strongyloidiasis in a patient with IgA deficiency. *Tropical gastroenterology : official journal of the Digestive Diseases Foundation* **1995**, 16 (4), 27-30.
47. Burke, J. A., Strongyloidiasis in childhood. *American journal of diseases of children (1960)* **1978**, 132 (11), 1130-6.
48. Berk, S. L., et al., Clinical and epidemiologic features of strongyloidiasis. A prospective study in rural Tennessee. *Archives of internal medicine* **1987**, 147 (7), 1257-61.
49. Ribeiro, L. C., et al., [Purpura in patient with disseminated strongyloidiasis]. *Rev Soc Bras Med Trop* **2005**, 38 (3), 255-7.
50. Weiser, J. A., et al., Periumbilical parasitic thumbprint purpura: strongyloides hyperinfection syndrome acquired from a cadaveric renal transplant. *Transpl Infect Dis* **2011**, 13 (1), 58-62.
51. Karanam, L. S., et al., Strongyloides stercoralis Hyper infection Syndrome. *Indian J Surg* **2021**, 83 (Suppl 3), 582-586.
52. Cho, J. Y., et al., [A case of steroid-induced hyperinfective strongyloidiasis with bacterial

- meningitis]. *Korean J Gastroenterol* **2012**, 60 (5), 330-4.
53. Sasaki, Y., et al., Meningitis associated with strongyloidiasis in an area endemic for strongyloidiasis and human T-lymphotropic virus-1: a single-center experience in Japan between 1990 and 2010. *Infection* **2013**, 41 (6), 1189-93.
 54. Shimasaki, T., et al., Five cases of recurrent meningitis associated with chronic strongyloidiasis. *Am J Trop Med Hyg* **2015**, 92 (3), 601-4.
 55. Zammarchi, L., et al., Persistent strongyloidiasis complicated by recurrent meningitis in an HTLV seropositive Peruvian migrant resettled in Italy. *Am J Trop Med Hyg* **2015**, 92 (6), 1257-60.
 56. Newberry, A. M., et al., *Strongyloides* hyperinfection presenting as acute respiratory failure and gram-negative sepsis. *Chest* **2005**, 128 (5), 3681-4.
 57. Arsic-Arsenijevic, V., et al., Fatal *Strongyloides stercoralis* infection in a young woman with lupus glomerulonephritis. *J Nephrol* **2005**, 18 (6), 787-90.
 58. Siddiqui, A. A.; Berk, S. L., Diagnosis of *Strongyloides stercoralis* infection. *Clin Infect Dis* **2001**, 33 (7), 1040-7.
 59. Jones, C. A.; Abadie, S. H., Studies in human strongyloidiasis. II. A comparison of the efficiency of diagnosis by examination of feces and duodenal fluid. *Am J Clin Pathol* **1954**, 24 (10), 1154-8.
 60. Costa-Cruz, J. M., et al., Cryo-microtome sections of coproculture larvae of *Strongyloides stercoralis* and *Strongyloides ratti* as antigen sources for the immunodiagnosis of human strongyloidiasis. *Rev Inst Med Trop Sao Paulo* **1997**, 39 (6), 313-7.
 61. Attia, M. M., et al., Some observations on coproculture of *Strongyloides stercoralis*. *J Egypt Soc Parasitol* **1982**, 12 (1), 103-6.
 62. van Doorn, H. R., et al., Use of enzyme-linked immunosorbent assay and dipstick assay for detection of *Strongyloides stercoralis* infection in humans. *J Clin Microbiol* **2007**, 45 (2), 438-42.
 63. Von Kuster, L. C.; Genta, R. M., Cutaneous manifestations of strongyloidiasis. *Arch Dermatol* **1988**, 124, 1826-1830.
 64. Buonfrate, D., et al., Accuracy of five serologic tests for the follow up of *Strongyloides stercoralis* infection. *PLoS Negl Trop Dis* **2015**, 9 (2), e0003491.
 65. Tamarozzi, F., et al., Accuracy, acceptability, and feasibility of diagnostic tests for the screening of *Strongyloides stercoralis* in the field (ESTRELLA): a cross-sectional study in Ecuador. *Lancet Glob Health* **2023**, 11 (5), e740-e748.
 66. Cimino, R. O., et al., Identification of human intestinal parasites affecting an asymptomatic peri-urban Argentinian population using multi-parallel quantitative real-time polymerase chain reaction. *Parasit Vectors* **2015**, 8, 380.
 67. Neva, F. A., Biology and immunology of human strongyloidiasis. *J Infect Dis* **1986**, 153 (3), 397-406.
 68. Naquira, C., et al., Ivermectin for human strongyloidiasis and other intestinal helminths. *Am J Trop Med Hyg* **1989**, 40 (3), 304-9.
 69. Lyagoubi, M., et al., Chronic persistent strongyloidiasis cured by ivermectin. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1992**, 86 (5), 541.
 70. Wijesundera, M. D., et al., Ivermectin therapy in chronic strongyloidiasis. *Trans Trop Med Hyg* **1994**, 291, 86.
 71. Gann, P. H., et al., A randomized trial of single- and two-dose ivermectin versus thiabendazole for treatment of strongyloidiasis. *J Infect Dis* **1994**, 169 (5), 1076-9.
 72. Muennig, P., et al., The cost-effectiveness of ivermectin vs. albendazole in the presumptive treatment of strongyloidiasis in immigrants to the United States. *Epidemiol Infect* **2004**, 132 (6), 1055-63.
 73. Segarra-Newnham, M., Manifestations, diagnosis, and treatment of *Strongyloides stercoralis* infection. *Ann Pharmacother* **2007**, 41 (12), 1992-2001.
 74. Mejia, R.; Nutman, T. B., Screening, prevention, and treatment for hyperinfection syndrome and disseminated infections caused by *Strongyloides stercoralis*. *Curr Opin Infect Dis* **2012**, 25 (4), 458-63.
 75. Boggild, A. K., et al., CATMAT statement on disseminated strongyloidiasis: Prevention, assessment and management guidelines. *Can Comm Dis Rep* **2016**, 42, 12-19.
 76. Schar, F., et al., *Strongyloides stercoralis* larvae excretion patterns before and after treatment.

- Parasitology* **2014**, *141* (7), 892-7.
77. Repetto, S. A., et al., Strongyloidiasis Outside Endemic Areas: Long-term Parasitological and Clinical Follow-up After Ivermectin Treatment. *Clin Infect Dis* **2018**, *66* (10), 1558-1565.
 78. Barrett, J.; Newsholme, W., Subcutaneous ivermectin use in the treatment of severe *Strongyloides stercoralis* infection: two case reports and a discussion of the literature-authors' response. *J Antimicrob Chemother* **2016**.
 79. Buonfrate, D., et al., Comment on: Subcutaneous ivermectin use in the treatment of severe *Strongyloides stercoralis* infection: two case reports and a discussion of the literature. *J Antimicrob Chemother* **2016**.
 80. Barnish, G.; Barker, J., An intervention study using thiabendazole suspension against *Strongyloides fuelleborni*-like infections in Papua New Guinea. *Trans R Soc Trop Med Hyg* **1987**, *81* (1), 60-3.
 81. Yori, P. P., et al., Seroepidemiology of strongyloidiasis in the Peruvian Amazon. *Am J Trop Med Hyg* **2006**, *74* (1), 97-102.
 82. Slenczka, W.; Klenk, H. D., Forty years of marburg virus. *J Infect Dis* **2007**, *196 Suppl 2*, S131-5.
 83. Nair, D., Screening for *Strongyloides* infection among the institutionalized mentally disabled. *J Am Board Fam Pract* **2001**, *14* (1), 51-3.
 84. Krolewiecki, A. J., et al., A public health response against *Strongyloides stercoralis*: time to look at soil-transmitted helminthiasis in full. *PLoS Negl Trop Dis* **2013**, *7* (5), e2165.
 85. Organization, W. H., Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva. Licence: CC BY-NC-SA 3.0 IGO **2020**.

21. *Trichinella spiralis*

(Railliet 1896)

Pronunciation: \trik-ə-'nel-ə\

Introduction

The genus *Trichinella* (\TRIK-in-el-ah\) consists of ten species and three genotypes, all with different geographical distributions.¹ Each is capable of infecting humans.²⁻¹⁰ *Trichinella* spp. include: *T. spiralis*, *T. britovi*, *T. pseudospiralis*, *T. papua*, *T. nativa*, *T. nelsoni*, *T. murrelli*, *T. zimbabwensis*, *T. patagoniensis*, and *T. chanchalensis*.^{2, 11-13} *Trichinella* spp. infect a broad spectrum of mammalian hosts, making them one of the world's most widely distributed groups of nematode infections. *Trichinella* spp. are members of the family Trichurata and are genetically related to *Trichuris trichiura* and *Capillaria* spp. *Trichinella* spp. constitute an unusual group of organisms in the phylum Nematoda in that they all live a part of their adult lives as intra-multicellular parasites. All *Trichinella* species are transmitted by ingesting raw or undercooked infected meats.

Trichinellosis is the name of the disease caused by *Trichinella* spp. Currently, the prevalence of trichinellosis is low within the United States, mainly occurring as scattered outbreaks, less often involving pork con-

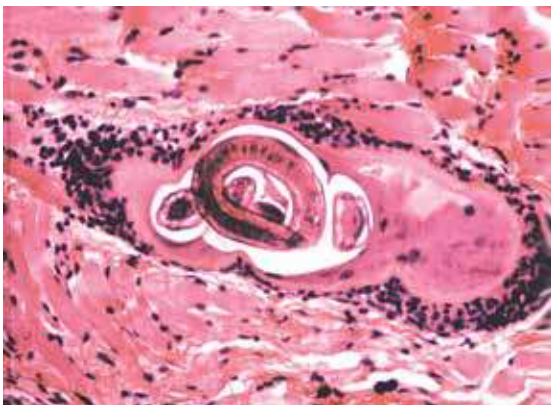


Figure 21.1. Infective first stage larva of *Trichinella spiralis* in its Nurse cell in muscle tissue. The worm measures 1mm x 36 μ m.

sumption and more often involving poorly cooked game, with most human cases being due to *Trichinella spiralis* and *T. murrelli*.^{10, 14-16} The domestic pig is the primary reservoir host for *T. spiralis*. This species is significantly more prevalent in people living in certain parts of Europe, Asia, and Southeast Asia than in the United States. It is now considered endemic in Japan and China. A large outbreak of trichinellosis occurred in Lebanon in 1997, infecting over 200 people.¹⁷ *T. spiralis* infection in humans was reported from Korea for the first time in 2000.¹⁸ In contrast, nematode infections in wildlife within the United States are now thought to be largely due to *T. murrelli*.¹⁹

An outbreak of *T. pseudospiralis* in Thailand was reported.²⁰ This species can also infect birds of prey. Foci have also been described in Sweden, the Slovak Republic, and Tasmania (Australia).²¹⁻²³ *Trichinella paupae*, similar in biology to *T. pseudospiralis*, has been described in wild and domestic pigs in Papua New Guinea.¹² A new species, *Trichinella chanchalensis* has recently been described from an isolate of wolverine muscle tissue.²⁴

Humans can also be infected with *T. nativa* and *T. britovi*.^{25, 26} Reservoir hosts for *T. nativa* include sled dogs, walruses, and polar bears. *T. britovi* is the sylvatic form of trichinellosis throughout most of Asia and Europe. Numerous reports in the literature of infections with this parasite in foxes, raccoons, dogs, opossums, domestic and wild dogs, and cats are reported.

T. nelsoni is restricted to mammals in Equatorial Africa, such as hyenas and the large predatory cats.²⁷ Occasionally, people acquire infection with *T. nelsoni*.²⁸ Most animals in the wild, regardless of their geographic location, acquire *Trichinella* by scavenging. *T. zimbabwensis* infects crocodiles and mammals in Africa and is a non-encapsulate species associated with pansteatitis (generalized

body fat inflammation) outbreaks in crocodile populations in many areas of Sub-Saharan Africa.^{12, 29} *T. pseudospiralis* has been isolated from the Tasmanian Devil but not from humans living in that part of Australia.²³

Historical Information

In 1821, the encysted larva was recognized in muscle but was not associated with human disease.³⁰ In 1835, a medical student at St. Bartholomew's Hospital in London, James Paget, discovered the worm in human muscle tissue.³¹ Richard Owen, the director of the British Museum of Natural History, also observed the worms in muscle tissue derived from the same cadaver from which Paget obtained his muscle biopsy. Owen published the report of this discovery.³⁰ Both Rudolf Virchow and Friedrich Zenker, in 1859 and 1860, independently described the adult stage of *Trichinella*.³² Zenker was the first to recognize that eating raw pork could result in human infection with *Trichinella*.³³

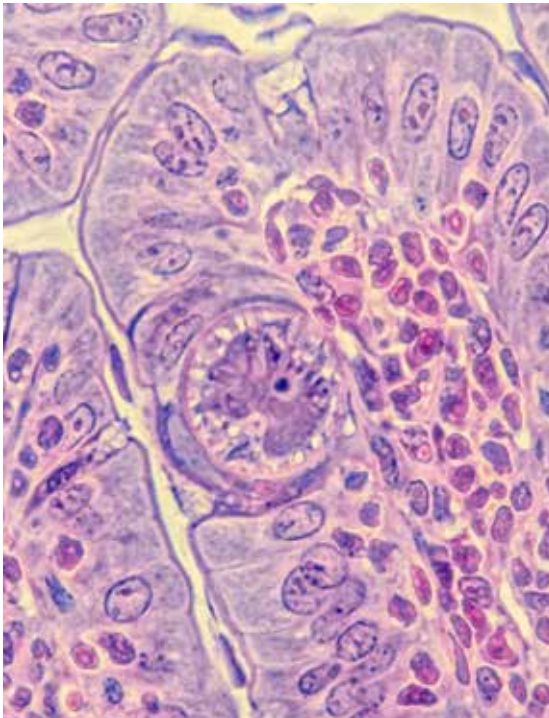


Figure 21.2. Adult *T. spiralis* in situ. Small intestine of experimentally infected mouse. The worm is embedded within the cytoplasm of the columnar cells.

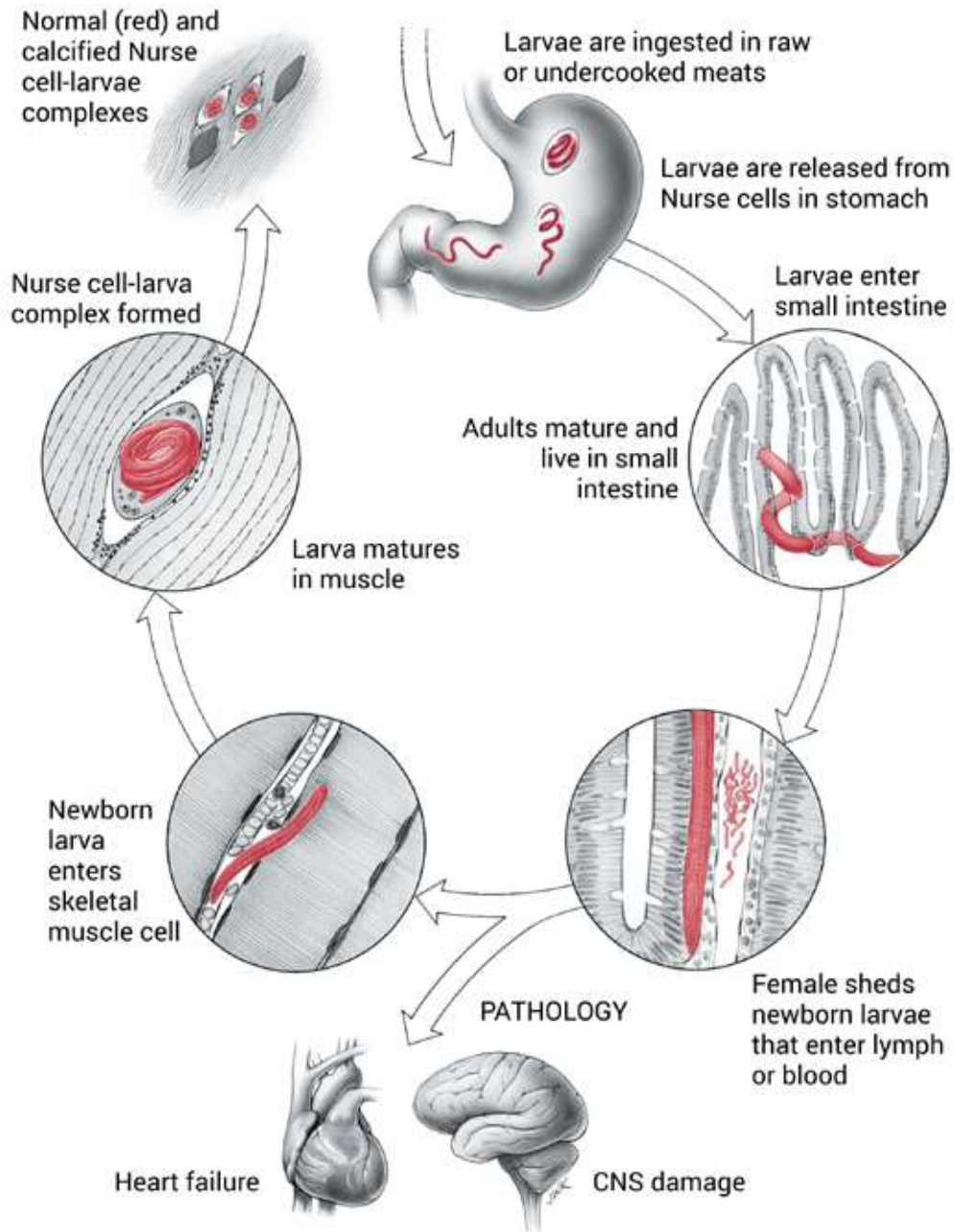
Life Cycle

Trichinella has both a domestic and a sylvatic cycle. The domestic cycle involves animals such as pigs and horses. The sylvatic cycle involves many wild animals, including wild boar, bear, moose, cougar, crocodile, fox, birds, and walrus.³⁴ In both cycles, infection is initiated by ingesting raw or undercooked meats harboring the Nurse cell-larva complex (Fig. 21.1). Larvae are released from muscle tissue by digestive enzymes in the stomach and then locate to the upper two-thirds of the small intestine. The outermost cuticular layer (epicuticle) becomes partially digested.^{35, 36} This enables the parasite to receive environmental cues, enabling it to select an infection site within the small intestine.³⁷ The immature parasites penetrate the columnar epithelium at the base of the villus. They live within a row of these cells and are now considered intra-multicellular organisms (Figs. 21.2, 21.7).³⁸



Figure 21.3. Adult female *T. spiralis*. 3 mm x 36 μ m. Note fully formed larvae in uterus.

Trichinella spiralis



Larvae rapidly molt four times over 30 hours, developing into adults. The female measures 3 mm by 36 μm (Fig. 21.3), while the male measures 1.5 mm by 36 μm (Fig. 21.4). Patency occurs within five days after mating. Adult females produce live offspring, L1 newborn larvae (Fig. 21.5), that measure 0.08 mm by 7 μm . The female produces offspring for as long as host immunity does not develop.³⁹ Acquired protective responses interfere with the overall process of embryogenesis and create physiological conditions in the local area of infection that force the adult parasites to relocate further down the intestinal tract. Expulsion of worms from the host is the final expression of immunity and may take several weeks.

The newborn larva is the parasite's only stage with a sword-like stylet in its oral cavity. It uses it to create an entry hole in potential host cells. Larvae enter the lamina propria in this fashion, penetrating either the mesenteric lymphatics or the bloodstream. Most new-



Figure 21.4. Adult male *T. spiralis*. Note claspers on tail (lower end). 1.5 mm x 36 μm .



Figure 21.5. Newborn larva of *T. spiralis*. 70 x 7 μm .

born larvae enter the general circulation and become distributed throughout the body.

Migrating newborns leave capillaries and enter cells (Fig. 21.6). There appears to be no tropism for any particular cell type. Once inside a cell, they can either remain or leave, depending upon environmental cues (yet to be determined) received by the parasite. Most cell types die as a result of invasion. Skeletal muscle cells are the exception.⁴⁰ Not only do the parasites remain inside them after invasion, but they induce a remarkable series of changes, causing the fully differentiated muscle cell to transform into one that supports the growth and development of the larva (Figs 21.8, 21.9). This process is termed Nurse cell formation.⁴¹ Parasite and its host cell develop in a coordinated fashion. *T. spiralis* is infective by the 14th day of infection, but the worm continues to grow in size until day 20.⁴² The biological significance of this precocious behavior has yet to be fully appreciated.

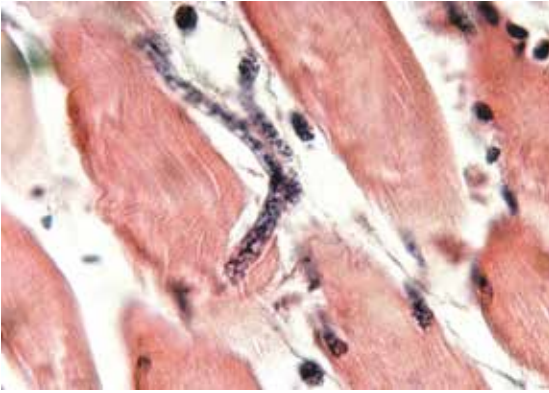


Figure 21.6. Newborn larva of *T. spiralis* entering muscle cell.

Parasites inside cells other than striated muscle cells fail to induce Nurse cells and either reenter the general circulation or die. Nurse cell formation creates an intimate and permanent association between the worm and its intracellular niche. At the cellular level, myofilaments and other related muscle cell components become replaced over a 14–16-day period by whorls of smooth membranes and clusters of dysfunctional mitochondria.

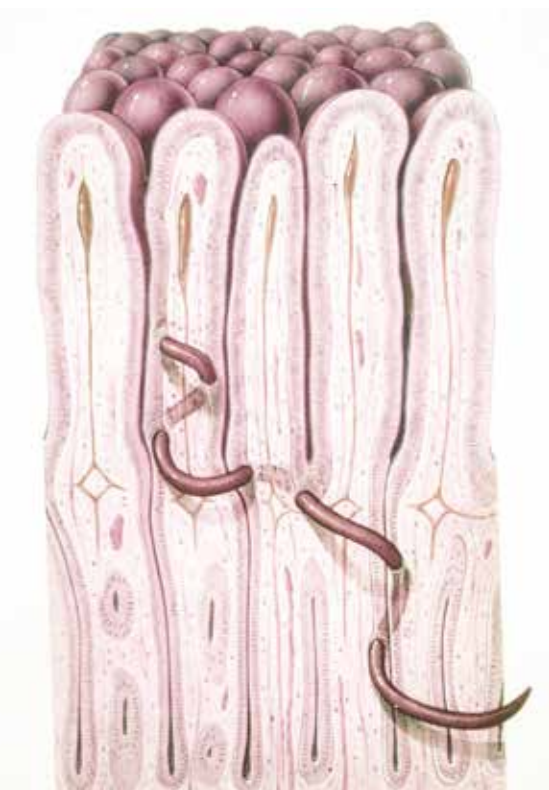


Figure 21.7. An adult female *T. spiralis*, depicted *in situ*. Drawing by J. Karapelou.

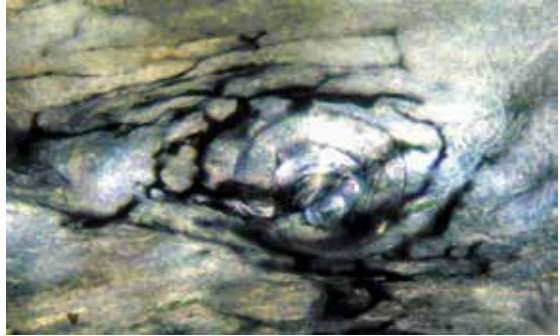


Figure 21.8. Nurse cell-parasite complex of *T. spiralis*, *in situ*. Infected mouse was injected with India ink to visualize circulatory rete.

The net result is that the host cell switches from an aerobic to an anaerobic metabolism.⁴³ Host cell nuclei enlarge and divide, amplifying the host's genome within the Nurse cell cytoplasm.^{44,45} The Nurse cell-parasite complex can live for as long as the host remains alive. Most do not and are calcified within several months after forming. Another mammal must eat infected host tissue for the life cycle to continue. Omnivorous and carnivorous modes are common among wild mammals, which helps maintain *T. spiralis* and its relatives in their respective host species.

Cellular and Molecular Pathogenesis

The enteral (intestinal) phase includes larval stages L1–L4 and the adult stage. In humans, this phase can last up to 3 weeks or more. Developing worms damage columnar epithelium, depositing shed cuticula there. Later in the infection, at the onset of the production of newborns, local inflammation, consisting of infiltration by eosinophils, neutrophils, and lymphocytes, intensifies in the local area. Villi flatten and become somewhat less absorbent, but not enough to result in malabsorption syndrome.

When larvae penetrate the lymphatic circulation or bloodstream, a bacteremia consist-



Figure 21.9. Nurse cell-parasite complex. Phase interference. Photo by E. Gravé.

ing of enteric flora may result, and cases of death due to sepsis have been reported. While *Trichinella* can induce polymicrobial bacteremia through violation of the gastrointestinal (GI) mucosal barrier, the excretory-secretory products of *Trichinella* may be protective against an overly robust host response by restraining MyD88 signaling via the mannose receptor.⁴⁶ Loss of wheat germ agglutinin receptors along the entire small intestine occurs.⁴⁷ The myenteric electric potential is interrupted during the enteral phase, resulting in a slow-down in gut motility.⁴⁷

The parenteral phase of infection induces most of the pathological consequences. It is parasitemia-dependent and is attributable directly to the migrating newborn larvae as they randomly penetrate cells (e.g., brain, liver, kidney, and heart) in their search for striated skeletal muscle cells (Fig. 21.6). Cell death is the usual result of these events. The more penetration events there are, the more severe the resulting pathology. Generalized edema results during heavy infection. Proteinuria may ensue. Cardiomyopathies and CNS abnormalities are also common in those experiencing moderate to heavy infection.²

Experimental infections in immunologically defined strains of rodents have shown that the total number of muscle larvae produced was dependent upon numerous factors related to

the immune capabilities of a given strain. The Induction of interleukin-4 and interleukin-13 and the production of eosinophils and IgE antibodies appear to be essential for limiting the production of newborn larvae and expelling adult worms.^{48, 49} While host factors may lead to the expulsion of adult worms and limit their production of newborn larvae, these same factors, interleukin-4, interleukin-13, and the influx of eosinophils to infected muscle cells, appear to be essential for the maturation of the Nurse-cell complex.⁵⁰ TNF-induced NO production is not one of the effector mechanisms since knock-out mice that cannot produce NO expelled their parasites in a typical fashion without local gut damage.⁵¹ In NO⁺ mice, the expulsion of adults was accompanied by cellular pathology surrounding the worms. Local production of NO during the development of inflammation may be a contributing factor to the development of intestinal pathology during infection with *Trichinella*.

Clinical Disease

Presentation of the disease varies over time and, as a result, resembles a wide variety of clinical conditions (Fig. 21.10).^{2, 52} Trichinellosis is often misdiagnosed for that reason. The severity of the disease is parasitemia-dependent, making the diagnosis based solely on symptoms difficult, at best. In severe cases, death may ensue. Signs and symptoms should alert the physician to include trichinellosis in the differential diagnosis.

While light infection with *Trichinella* can pass unnoticed with minimal or no identifiable symptoms, heavy infection with many viable larvae presents a distinct two-stage syndrome.⁸ The first few days of the infection are characterized by the GI stage with gastroenteritis associated with secretory diarrhea, abdominal pain, and vomiting.⁵³

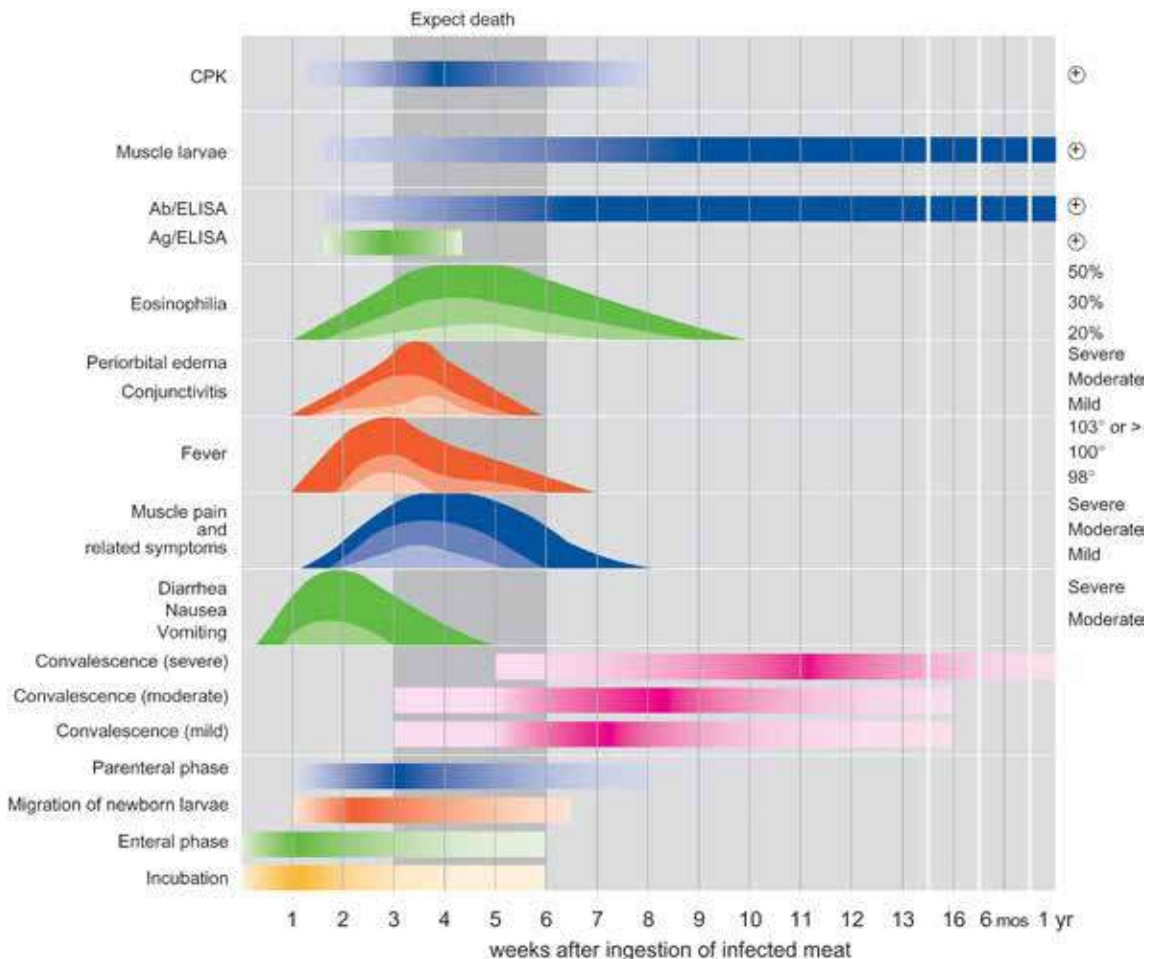


Figure 21.10. Summary of clinical correlations. The degree of manifestation of signs and symptoms is dependent upon the dose of larvae ingested. The stages of the parasite and the signs and symptoms associated with them are shown in the same colors.

This transitory phase abates within ten days after ingesting infected tissue. A history of eating raw or undercooked meats helps rule out this parasitic infection. Others who also ate the same meats and are suffering similarly reinforce the suspicion of trichinellosis. Many clinicians mistakenly opt for a food poisoning scenario at this juncture.

The parenteral phase begins approximately one week after infection and may last several weeks. Typically, the patient has fever and myalgia, bilateral periorbital edema, and petechial hemorrhages, which are seen most clearly in the subungual region but are also observable in the conjunctivae and other mucosal sites.⁵⁴ Muscle tenderness can be

readily detected. Laboratory studies reveal a moderately elevated white blood cell count (12,000–15,000 cells/mm³) and a circulating eosinophilia ranging from 5% to as high as 50% (usual absolute eosinophil range 600–7,500 cells/mm³).^{55, 56}

Larvae penetrating tissues other than skeletal muscle may give rise to severe sequelae. Cardiovascular involvement may lead to myocarditis in many cases of moderate to severe infection. This aspect has been overrated as a clinical feature typical of most infections with this parasite since most instances encountered by the clinician are of a mild variety.⁵⁷ Electrocardiographic changes can occur during this phase, even without symp-

toms. Parasite invasion of the diaphragm and the accessory muscles of respiration may result in dyspnea.⁵⁸ Neurotrichinellosis occurs in association with invasion of the CNS.⁵⁹ In up to 25% of patients with heavy infection, meningitis and encephalitis can develop with edema, infarcts, and hemorrhage in fatal cases.^{60, 61} The convalescent phase follows the acute phase, during which time many, but not all, Nurse-cell-parasite complexes die.

Two clinical presentations have been described for *T. nativa* infections resulting from ingesting infected polar bear or walrus meat: a classic myopathic form and a second form presenting as a persistent diarrheal illness. The second form represents a secondary infection in previously sensitized individuals.⁶² The amount of ingested larvae, the species of *Trichinella*, and the status of the infected host impact the clinical presentation.⁶³

Diagnosis (see Clinical Appendix)

Definitive diagnosis depends upon finding the Nurse cell-parasite complex in muscle biopsy by microscopic examination (Fig 21.1) or detecting *Trichinella*-specific DNA.⁶⁴ PCR is sensitive and specific for detecting small numbers of larvae in muscle tissue but is rarely used in the clinical setting.⁵⁴ Real-time PCR has been developed and is used in surveillance studies of wildlife *Trichinella* infection.⁶⁵

Muscle biopsy can be negative, even in the heaviest of infections, due to sampling errors. As a consequence, it is recommended that symptomatic muscle, preferentially a more proximal muscle such as the deltoid, near the tendinous insertion, be sampled to improve sensitivity.² Also, the larvae may be at an early stage of their development, making them inconspicuous, even to experienced patholo-

gists. Serological tests are available with confirmatory Western blot testing.⁶⁶ Serological tests begin to show positive results within two weeks. ELISA can detect antibodies in some patients as early as 12 days after infection. Muscle enzymes, such as creatine phosphokinase (CPK) and lactic dehydrogenase, are released into the circulation, causing an increase in their serum levels.

A rising, plateauing, and falling level of circulating eosinophils throughout the infection period is not direct proof of infection, but with this information and a history of exposure, the clinician could treat the patient presumptively. It is helpful to remember that wild mammals can also be sources of infection. Outbreaks of trichinellosis have been traced to hunters and the recipients of their kills.⁶⁷⁻⁶⁹

Treatment (see Clinical Appendix)

While many cases of *Trichinella* infection may be mild or even asymptomatic and easily managed with analgesics and antipyretics, more significant infections are usually managed with antihelminthic therapy and corticosteroids.⁵⁴ Adult worms may still be producing larvae when patients present with symptoms. Treatment with albendazole or mebendazole may be beneficial. Treatment with mebendazole early in infection has been associated with decreased muscle pain and inflammation...⁷⁰ Once muscle invasion has occurred, treatment with these agents is useless.⁷¹ Anti-inflammatory corticosteroids, particularly prednisolone, are recommended if the diagnosis is secure.^{2, 51} The myopathic phase is treated with antipyretics and analgesics (aspirin, acetaminophen) and should be continued until the fever and allergic signs recede. Because of their immunosuppressive potential, steroids should be administered with caution.

Prevention and Control

Within the last ten years, outbreaks of trichinellosis in the United States have been rare.^{14-16, 72} Most have been associated with ingesting raw or undercooked meats from game animals and not from commercial sources.¹⁴⁻¹⁶ This represents a shift in the epidemiology of outbreaks compared to 30-40 years ago when commercial sources of infected pork were much more common than today. Compared to commercial farm operations, pigs raised on individual farms are likelier to be fed uncooked garbage and acquire the infection. This is because feeding unprocessed garbage containing meat scraps is against federally mandated regulations. In the past ten years, small farms have, in the main, been bought up and replaced with larger so-called “factory” farms, in which upwards of 10,000 pigs can be managed with a minimum of labor. Enforcement of laws governing the running of large production facilities is intensive and has been key in reducing the spread of diseases infecting livestock and humans.⁷²

Top carnivores like bear, fox, and cougar often become infected. Hunters and those sharing their kill are best warned to cook all meat thoroughly. Herbivores can also harbor the infection since most plant eaters occasionally ingest meat when the opportunity arises. Epidemics due to eating raw horse-meat have been reported in France, Italy, and Poland.⁷³

Meat inspection for the detection of *Trichinella* is nonexistent in the United States. In Europe, the countries participating in the common market employ several strategies for examining meat for muscle larvae. Most serve to identify pools of meat samples from given regions. A *Trichinella*-free designation is applied to that meat supply if they are consistently negative. Rare outbreaks occur despite this rigorous system of inspection.

Trichinellosis due to *T. spiralis* derived from pork can be prevented by cooking meat thoroughly at 58.5 °C for 10 minutes or freezing it at -20 °C for three days. Freezing is not always effective with meat from other animals or for other species of *Trichinella*. For example, *Trichinella* in bears and raccoons may survive at temperatures below freezing.⁷⁴ *T. nativa* was found to survive for up to 34 months in frozen muscle tissue of grizzly bears and up to 5 years frozen at -18 °C in the muscles of various wild species of carnivores.⁶ It appears that the freeze tolerance of encapsulated *Trichinella* muscle larva is largely determined by which *Trichinella* spp. is involved but may also depend on aspects of host tissue.⁷⁴⁻⁷⁶ Thorough cooking, achieving an internal temperature of 58.5 °C for 10 minutes, is the only way to render all meats safe from all *Trichinella* species.

References

1. Zarlenga, D., et al., *Trichinella* species and genotypes. *Research in veterinary science* **2020**, 133, 289-296.
2. Bruschi, F.; Murrell, K. D., New aspects of human trichinellosis: the impact of new *Trichinella* species. *Postgraduate medical journal* **2002**, 78 (915), 15-22.
3. Rombout, Y. B., et al., Detection and identification of eight *Trichinella* genotypes by reverse line blot hybridization. *Journal of clinical microbiology* **2001**, 39 (2), 642-6.
4. Zarlenga, D. S., et al., A multiplex PCR for unequivocal differentiation of all encapsulated and non-encapsulated genotypes of *Trichinella*. *Int J Parasitol* **1999**, 29 (11), 1859-67.
5. Appleyard, G. D., et al., Differentiation of *Trichinella* genotypes by polymerase chain reaction

- using sequence-specific primers. *J Parasitol* **1999**, 85 (3), 556-9.
6. Pozio, E.; Zarlenga, D. S., Recent advances on the taxonomy, systematics and epidemiology of *Trichinella*. *Int J Parasitol* **2005**, 35 (11-12), 1191-204.
7. Murrell, K. D., et al., The systematics of the genus *Trichinella* with a key to species. *Vet Parasitol* **2000**, 93 (3-4), 293-307.
8. Murrell, K. D.; Bruschi, F., Clinical trichinellosis. *Prog Clin Parasitol* **1994**, 4, 117-50.
9. Murrell, K. D.; Pozio, E., Trichinellosis: the zoonosis that won't go quietly. *Int J Parasitol* **2000**, 30 (12-13), 1339-49.
10. Korhonen, P. K., et al., Phylogenomic and biogeographic reconstruction of the *Trichinella* complex. *Nat Commun* **2016**, 7, 10513.
11. Pozio, E., Foodborne and waterborne parasites. *Acta Microbiol Pol* **2003**, 52 Suppl, 83-96.
12. Pozio, E., et al., *Trichinella zimbabwensis* n.sp. (Nematoda), a new non-encapsulated species from crocodiles (*Crocodylus niloticus*) in Zimbabwe also infecting mammals. *International journal for parasitology* **2002**, 32 (14), 1787-99.
13. Krivokapich, S. J., et al., *Trichinella patagoniensis* n. sp. (Nematoda), a new encapsulated species infecting carnivorous mammals in South America. *Int J Parasitol* **2012**, 42 (10), 903-10.
14. Wilson, N. O., et al., Trichinellosis surveillance--United States, 2008-2012. *Morbidity and mortality weekly report. Surveillance summaries* **2015**, 64 Suppl 1, 1-8.
15. Greene, Y. G., et al., Trichinellosis caused by consumption of wild boar meat - Illinois, 2013. *MMWR Morb Mortal Wkly Rep* **2014**, 63 (20), 451.
16. Moorehead, A., et al., Trichinellosis in the United States, : declining but not gone. *Am Med Hyg* **1999**, 60, 1991-1996.
17. Haim, M., et al., An outbreak of *Trichinella spiralis* infection in southern Lebanon. *Epidemiology and infection* **1997**, 119 (3), 357-62.
18. Sohn, W. M., et al., The first human case of *Trichinella spiralis* infection in Korea. *The Korean journal of parasitology* **2000**, 38 (2), 111-5.
19. Pozio, E.; La Rosa, G., *Trichinella murrelli* n. sp: etiological agent of sylvatic trichinellosis in temperate areas of North America. *J Parasitol* **2000**, 86 (1), 134-9.
20. Jongwutiwes, S., et al., First outbreak of human trichinellosis caused by *Trichinella pseudospiralis*. *Clin Inf Dis* **1998**, 26 (1), 111-5.
21. Pozio, E., et al., *Trichinella pseudospiralis* foci in Sweden. *Vet Parasitol* **2004**, 125 (3-4), 335-42.
22. Hurnikova, Z.; Snabel, V., First record of *Trichinella pseudospiralis* in the Slovak Republic found in domestic focus. *Vet Parasitol* **2005**, 128, 91-8.
23. Obendorf, D. L., et al., *Trichinella pseudospiralis* infection in Tasmanian wildlife. *Australian veterinary journal* **1990**, 67 (3), 108-10.
24. Sharma, R., et al., Hiding in plain sight: discovery and phylogeography of a cryptic species of *Trichinella* (Nematoda: Trichinellidae) in wolverine (*Gulo gulo*). *Int J Parasitol* **2020**, 50 (4), 277-287.
25. Pozio, E.; Kapel, C. M., *Trichinella nativa* in sylvatic wild boars. *Journal of helminthology* **1999**, 73 (1), 87-9.
26. Pozio, E., et al., Distribution of sylvatic species of *Trichinella* in Estonia according to climate zones. *J Parasitol* **1998**, 84 (1), 193-5.
27. La Rosa, G.; Pozio, E., Molecular investigation of African isolates of *Trichinella* reveals genetic polymorphism in *Trichinella nelsoni*. *International journal for parasitology* **2000**, 30 (5), 663-7.
28. Mukaratirwa, S., et al., *Trichinella* infections in animals and humans in sub-Saharan Africa: a review. *Acta Trop* **2013**, 125 (1), 82-9.
29. La Grange, L. J., et al., The occurrence of *Trichinella zimbabwensis* in naturally infected wild crocodiles (*Crocodylus niloticus*) from the Kruger National Park, South Africa. *J Helminthol* **2013**, 87 (1), 91-6.
30. Cox, F. E., History of human parasitology. *Clin Microbiol Rev* **2002**, 15 (4), 595-612.
31. Owens, R., Description of a microscopic entozoon infesting the muscles of the human body. *London Med. Gaz.* **1835**, 16, 125-127.
32. Virchow, R., Recherches sur le developpement de la *Trichina spiralis* (ce ver devient adulte dans l'intestin du chien). *C. R. Seanc. Acad. Sci.* **1859**, 49, 660-662.

33. Zenker, F. A., Ueber die Trichinen-krankheit des Menschen. *Arch. Pathol. Anat. Physiol. Klin. Med.* **1860**, 18, 561-572.
34. Pozio, E., Factors affecting the flow among domestic, synanthropic and sylvatic cycles of *Trichinella*. *Vet Parasitol* **2000**, 93 (3-4), 241-62.
35. Stewart, G. L., et al., *Trichinella spiralis*: behavior, structure, and biochemistry of larvae following exposure to components of the host enteric environment. *Experimental parasitology* **1987**, 63 (2), 195-204.
36. Modha, J., et al., The surface coat of infective larvae of *Trichinella spiralis*. *Parasitology* **1999**, 118 (Pt 5), 509-22.
37. Despommier, D., Behavioral cues in migration and location of parasitic nematodes, with special emphasis on *Trichinella spiralis*. 1982; p 110-126.
38. Wright, K. A., *Trichinella spiralis*: an intracellular parasite in the intestinal phase. *J Parasitol* **1979**, 65 (3), 441-5.
39. Howitt, M. R., et al., Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* **2016**, 351 (6279), 1329-33.
40. Despommier, D. D., *Trichinella spiralis* and the concept of niche. *J Parasitol* **1993**, 79 (4), 472-82.
41. Despommier, D. D., How does *Trichinella spiralis* make itself at home? *Parasitology today (Personal ed.)* **1998**, 14 (8), 318-23.
42. Despommier, D., et al., *Trichinella spiralis*: growth of the intracellular (muscle) larva. *Experimental parasitology* **1975**, 37 (1), 108-16.
43. Despommier, D. D.; Campbell, W. C., *Trichinella* and trichinellosis. 1983; p 75-152.
44. Despommier, D., et al., Changes in nurse cell nuclei during synchronous infection with *Trichinella spiralis*. *J Parasitol* **1991**, 77 (2), 290-5.
45. Jasmer, D. P., *Trichinella spiralis* infected skeletal muscle cells arrest in G2/M and cease muscle gene expression. *The Journal of cell biology* **1993**, 121 (4), 785-93.
46. Du, L., et al., *Trichinella spiralis* excretory-secretory products protect against polymicrobial sepsis by suppressing MyD88 via mannose receptor. *Biomed Res Int* **2014**, 2014, 898646.
47. Castro, G. A., et al., Pathophysiology of the gastrointestinal phase. 1983; p 209-241.
48. Finkelman, F. D., et al., Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. *Immunological reviews* **2004**, 201, 139-55.
49. Bell, R. G., The generation and expression of immunity to *Trichinella spiralis* in laboratory rodents. *Advances in parasitology* **1998**, 41, 149-217.
50. Huang, L., et al., Eosinophils and IL-4 Support Nematode Growth Coincident with an Innate Response to Tissue Injury. *PLoS Pathog* **2015**, 11 (12), e1005347.
51. Lawrence, C. E., et al., Nitric oxide mediates intestinal pathology but not immune expulsion during *Trichinella spiralis* infection in mice. *Journal of immunology (Baltimore, Md. : 1950)* **2000**, 164 (8), 4229-34.
52. Pozio, E., et al., Clinical aspects, diagnosis and treatment of trichinellosis. *Expert review of anti-infective therapy* **2003**, 1 (3), 471-82.
53. Kalia, N., et al., Intestinal secretory and absorptive function in *Trichinella spiralis* mouse model of postinfective gut dysfunction: role of bile acids. *Gut* **2008**, 57 (1), 41-9.
54. Gottstein, B., et al., Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clin Microbiol Rev* **2009**, 22 (1), 127-45, Table of Contents.
55. Bruschi, F., et al., Eosinophils and *Trichinella* infection: toxic for the parasite and the host? *Trends Parasitol* **2008**, 24 (10), 462-7.
56. Vu Thi, N., et al., The hidden burden of trichinellosis in Vietnam: a postoutbreak epidemiological study. *Biomed Res Int* **2013**, 2013, 149890.
57. Lazarevic, A. M., et al., Low incidence of cardiac abnormalities in treated trichinosis: a prospective study of 62 patients from a single-source outbreak. *Am* **1999**, 107, 18-23.
58. Compton, S. J., et al., Trichinosis with ventilatory failure and persistent myocarditis. *Clin Infect Dis* **1993**, 16 (4), 500-4.
59. Bruschi, F., et al., Neurotrichinellosis. *Handb Clin Neurol* **2013**, 114, 243-9.
60. Gelal, F., et al., Diffusion-weighted and conventional MR imaging in neurotrichinosis. *Acta*

- Radiol* **2005**, 46 (2), 196-9.
61. Mawhorter, S. D.; Kazura, J. W., Trichinosis of the central nervous system. *Semin Neurol* **1993**, 13 (2), 148-52.
 62. MacClean, J. D., et al., Epidemiologic and serologic definition of primary and secondary trichinosis in the arctic. *Dis* **1992**, 165, 908-912.
 63. Kociecka, W., Trichinellosis: human disease, diagnosis and treatment. *Vet Parasitol* **2000**, 93 (3-4), 365-83.
 64. Wu, Z., et al., Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for the identification of *Trichinella* isolates. *Parasitology* **1999**, 118 (Pt 2), 211-8.
 65. Cuttell, L., et al., Real-time PCR as a surveillance tool for the detection of *Trichinella* infection in muscle samples from wildlife. *Vet Parasitol* **2012**, 188 (3-4), 285-93.
 66. Moskwa, B., et al., The comparison of different ELISA procedures in detecting anti-*Trichinella* IgG in human infections. *Vet Parasitol* **2009**, 159 (3-4), 312-5.
 67. Garcia, E.; Mora, L., First record of human trichinosis in Chile associated with consumption of wild boar (*Sus scrofa*). *Mem Inst Oswaldo Cruz* **2005**, 100.
 68. CDC, Trichinellosis associated with bear meat-New York and Tennessee. *MMWR* **2004**, 53, 606-10.
 69. Rah, H., et al., Serosurvey of selected zoonotic agents in polar bears (*Ursus maritimus*). *The Veterinary record* **2005**, 156 (1), 7-13.
 70. Watt, G., et al., Blinded, placebo-controlled trial of antiparasitic drugs for trichinosis myositis. *J Infect Dis* **2000**, 182 (1), 371-4.
 71. Pozio, E., et al., Failure of mebendazole in the treatment of humans with *Trichinella spiralis* infection at the stage of encapsulating larvae. *Clin Infect Dis* **2001**, 32 (4), 638-42.
 72. Roy, S. L., et al., Trichinellosis surveillance--United States, 1997-2001. *Morbidity and mortality weekly report. Surveillance summaries (Washington, D.C. : 2002)* **2003**, 52 (6), 1-8.
 73. Murrell, K. D., et al., Epidemiology of *Trichinella* infection in the horse: the risk from animal product feeding practices. *Vet Parasitol* **2004**, 123 (3-4), 223-33.
 74. Kapel, C. M., et al., Freeze tolerance, morphology, and RAPD-PCR identification of *Trichinella nativa* in naturally infected arctic foxes. *J Parasitol* **1999**, 85 (1), 144-7.
 75. Lacour, S. A., et al., Freeze-tolerance of *Trichinella* muscle larvae in experimentally infected wild boars. *Vet Parasitol* **2013**, 194 (2-4), 175-8.
 76. Theodoropoulos, G., et al., Infectivity, predilection sites, and freeze tolerance of *Trichinella* spp. in experimentally infected sheep. *Parasitol Res* **2000**, 86 (5), 401-5.

22. Lymphatic Filariae

Wuchereria bancrofti

(Cobbold 1877)

Pronunciation: \wùk-ə-'rìr-ē-ə\ \ban-kròft-ī\

Brugia malayi

(Brug 1927)

Pronunciation: \brü- jē-ə\ \ma-lē-ī\

Brugia timori

(Partono 1977)

Pronunciation: \brü- jē-ə\ \tē-,mòr\

Introduction

Three species of vector-born nematodes cause lymphatic filariasis (LF) in humans: *Wuchereria bancrofti* (\WOOK-ah-rer-EE-ah\ban-KROFT-ee\), *Brugia malayi* (\BREW-gee-uh\MAH-la-eye\), and *B. timori* (\BREW-gee-uh\TEE-more-ee\).¹ These are thread-like nematodes whose adult stage lives within the lumen of lymphatic vessels.² At the turn of the century, more than 100 million people in Southeast Asia, Africa, and the Americas were infected with filariasis, with more than 90% due to *W. bancrofti*

infection.^{3,4} The remainder of the people were infected with *B. malayi*, while *B. timori* was restricted to the islands of Timor and Flores in southeastern Indonesia.⁵ The 2020 WHO NTD Roadmap estimates of disease burden includes approximately 51.4 million people infected in 2018 with 859 million people living in endemic areas requiring mass drug treatment to prevent further transmission.⁶ Elephantiasis, a disfiguring disease caused by blockage of the lymphatic vessels, affects large numbers of individuals living in endemic areas. The worms are ovoviviparous, producing larvae called microfilariae. Lymphatic filariasis is transmitted by culicine and anopheline species of mosquitoes.

Humans are the only host for *W. bancrofti*. The infection is widely distributed in the tropics, especially in South Asia, Africa (including Egypt), and tropical regions of the Americas. The major vectors are culicine mosquitoes in most urban and semi-urban areas, anophelines in rural areas of Africa and elsewhere, and *Aedes* spp. in the Pacific Islands. Except in the South Pacific, most of the *W. bancrofti* strains are nocturnal, referring to the periodicity with which the microfilariae appear in the peripheral circulation.

Infection with *B. malayi* is a zoonosis, with both feline and monkey reservoirs. *Mansonia* spp. serve as the major mosquito vector, although anophelines are sometimes involved in transmission. *B. malayi* infections occur in India, Malaysia, and Southeast Asia. Other minor members of the genus *Brugia* cause disease in humans, including *B. timori*, and accidental zoonotic *Brugia* infections (e.g., *B. beaveri* and *B. lepori*) that occur sporadically in the United States.^{7,8}

A major global effort was initiated to eliminate LF by the year 2020, based on the success of reducing the age-adjusted prevalence, but this has not been achieved and an updated

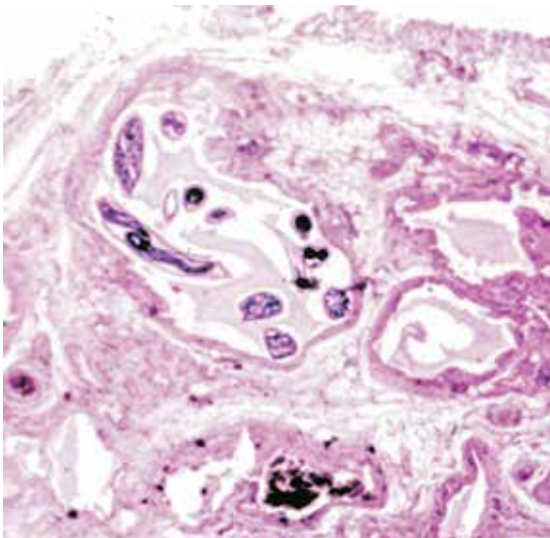
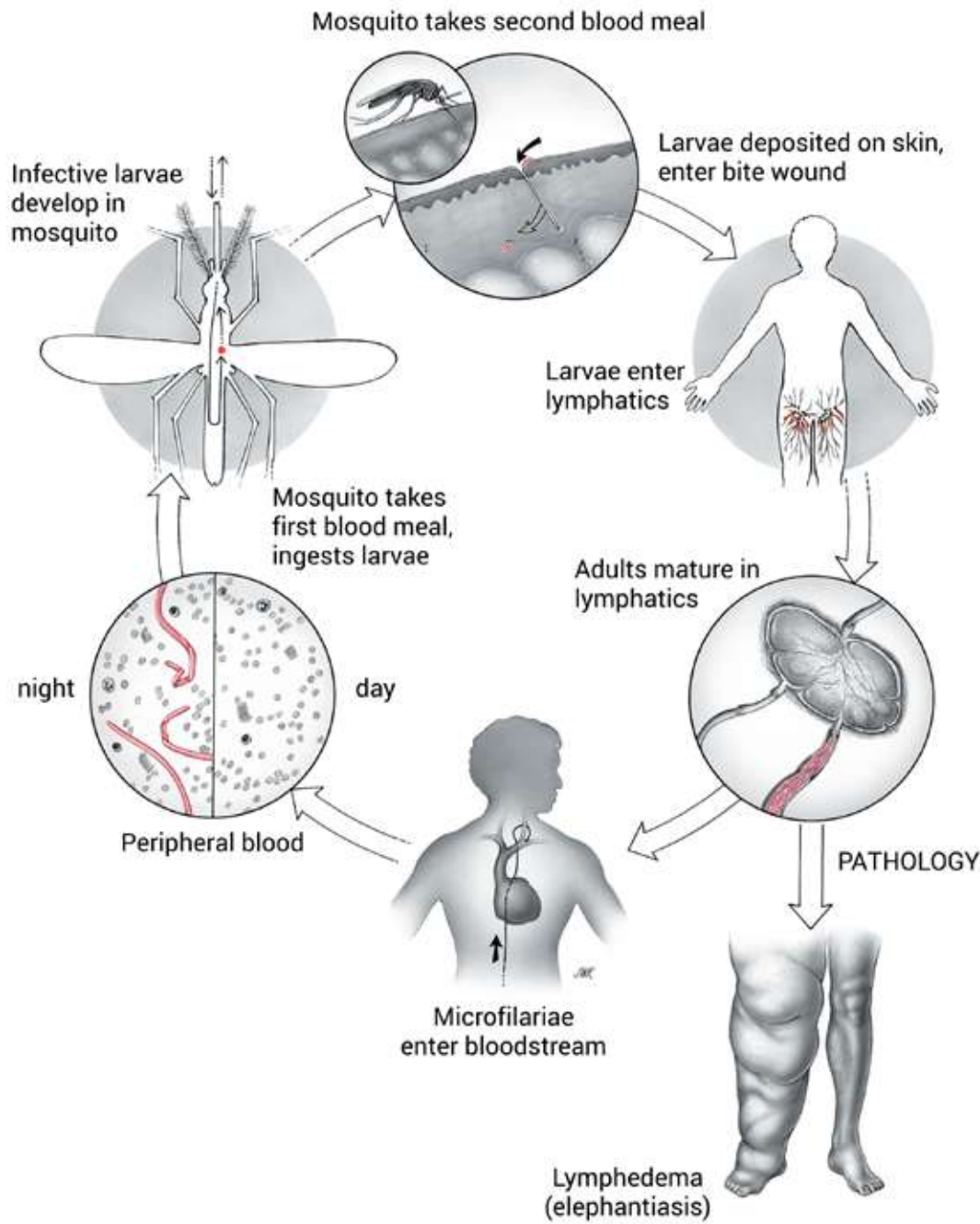


Figure 22.1. Adults of *Wuchereria bancrofti* in lymphatic vessels.

Wuchereria bancrofti



plan for elimination by 2030 is outlined in the 2020 WHO NTD Roadmap.^{6, 9-11} The term ‘elimination’ refers to the reduction of disease incidence to zero or close to zero in defined geographic areas, with a requirement for ongoing control efforts. At the same time, ‘eradication’ is the complete and permanent worldwide reduction to zero new disease cases.¹² The strategy for LF elimination relies on interrupting mosquito transmission by mass administration of combination therapy in endemic regions to reduce the number of microfilariae circulating in the bloodstream of infected individuals.

Historical Information

In 1863, Jean-Nicolas Demarquay, a French surgeon, described microfilariae of *Wuchereria* in hydrocele fluid.¹³ In 1866, Otto Wucherer described the microfilariae in urine in Brazil.¹⁴ In 1872, a Scottish physician working in Calcutta, Timothy Lewis, confirmed the presence of the microfilariae in urine and blood and recognized the connection with the severe lymphedema and deformities known as elephantiasis.^{15, 16} In 1877, Spencer Cobbold wrote a description of the adult worm and named the worm *Filaria bancrofti* in honor of Joseph Bancroft, the surgeon who had investigated the causes of hydrocele and lymphatic disease in Australia.^{16, 17} Lewis described the adult worm in India that same year.¹⁸ In 1878, Patrick Manson completed the life cycle description while working in Amoy (now called Xiamen) along the Chinese coast in Fujian Province.¹⁹ Today, LF has been largely eradicated from China.

Manson first demonstrated that mosquitoes were intermediate hosts for the parasite. For two decades, Manson maintained that infection was acquired when individuals drank water contaminated with larvae released from dead or dying mosquitoes. Eventually,

he accepted the concept that an infected mosquito’s bite transmitted the larvae. Filariasis may also be a water-borne disease under some circumstances since the oral route can induce experimental infections.²⁰

One of the most important developments in the history of LF control was the discovery by Francis Hawking and others that it is possible to reduce the prevalence of LF through mass treatment or, in some cases, by fortification of salt with diethylcarbamazine citrate (DEC).^{21, 22} During the 1970s and 1980s, Chinese parasitologists scaled this approach to the national level. It was achieved primarily through the fortification of regional salt supplies with DEC. The LF life cycle was discovered in China, and the parasite was first eliminated there. The accomplishments of the Chinese provide proof of the principle that it might be possible to eliminate LF worldwide through similar measures.

Life Cycle

Adult worms occupy the lumen of lymphatic vessels (Fig. 22.1) and have been found at all sites within the lymphatic circulation. Most commonly, they live in the lymphatics of the lower and upper extremities and male genitalia. Adult females are 3–10 cm in



Figure 22.2. Microfilaria of *W. bancrofti*. 250 μ m

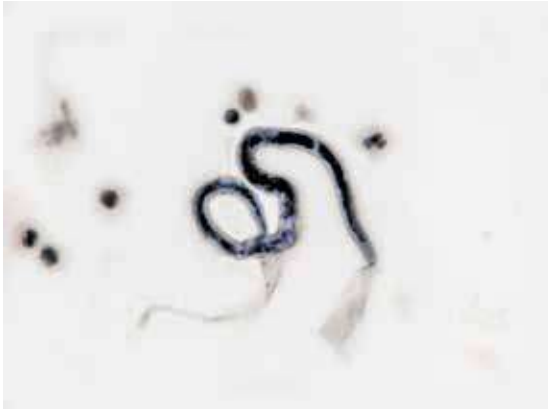


Figure 22.3. Microfilaria of *Brugia malayi*. 220 μ m

length, while the adult males are about half this length. Adult *Brugia* females are smaller, measuring 4–5.5 cm in length, with adult *Brugia* males being less than half this length. After mating, the female worm releases 10,000 or more offspring per day. Instead of releasing eggs, the worms release L1 larvae (microfilariae). Each microfilaria (Figs. 22.2, 22.3) measures approximately 270 μ m by 10 μ m and contains nuclei that characteristically do not extend to the tip of the tail. Another distinguishing feature is that the microfilaria is encased in a sheath comprised of chitin, a remnant of its eggshell.

Microfilariae migrate from the lymphatic circulation into the bloodstream. In most endemic areas worldwide, they are typically present in large numbers in the peripheral blood only at night (22:00–06:00). During the day, the microfilariae aggregate in the capillaries of the lungs when the activity of the host is increased (i.e., during strenuous exercise). It is speculated that nocturnal periodicity results from the microfilaria's preference for low oxygen tension. They are found in the peripheral bloodstream then, most likely in response to subtle pH changes in the pulmonary venous circulation during sleep.²³ In support of this hypothesis, experiments in which the sleep habits of infected volunteers were reversed also reversed the periodicity of microfilariae. The diurnal periodicity pat-

tern characteristic of the South Pacific strain has not been satisfactorily explained. Microfilariae live for about 1.5 years and must be ingested by a mosquito to continue their life cycle.

W. bancrofti is transmitted by a wide variety of mosquito genera and species, the most important being *Culex pipiens quinquefasciatus*, *C. pipiens pipiens*, *Anopheles gambiae*, and *A. polynesiensis*. *Aedes aegypti*, the yellow fever mosquito, can also transmit the infection in some Pacific Islands. Ingested microfilariae penetrate the stomach wall of the female mosquito and locate to the thoracic flight muscles. After maturing to the L1 stage (without molting), they undergo two molts, developing into L3 larvae, and are infective after 10–20 days of growth and development in the insect muscle tissue.

Infective L3 larvae migrate to the infected female mosquito's biting mouthparts and are deposited onto the skin adjacent to the bite wound during consumption of a subsequent blood meal. When the mosquito withdraws her mouthparts, larvae crawl into the open wound. Immature worms migrate through the subcutaneous tissues to the lymphatic vessels and locate near the draining lymph nodes of each vessel. The worms slowly develop into mature adults, which takes about 1 year. Soon after copulation, the gravid adult female worms begin shedding microfilariae. The longevity of adults, measured by the continuous production of microfilariae, is estimated at 5–8 years, but infections lasting 40 years have been reported.²⁴

The adult and larval stages of *B. malayi* resemble those of *W. bancrofti*. The life cycles of the two species of filariae are similar, although animal reservoirs occur for some members of the genus *Brugia*. The sub-periodic strain of *B. malayi* is a zoonosis acquired from forest monkeys and other wild

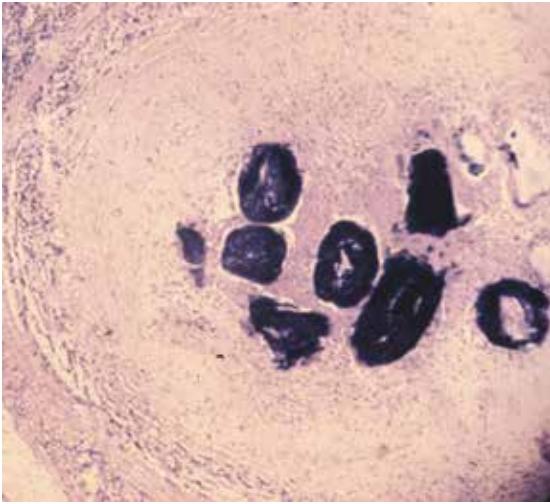


Figure 22.4. Calcified adults of *W. bancrofti* in blocked lymphatic vessel.

animals and transmitted through the bite of *Mansonia* spp. mosquitoes.⁷

Cellular and Molecular Pathogenesis

The pathogenesis of lymphangitis leading to elephantiasis has not been fully explained. It may result from a sequence of host-mediated immunopathologic events that occur in response to dead and dying adults within the lymphatics (Fig. 22.4). In contrast, living adult worms or the microfilariae are believed to suppress these responses, and typically adult worms do not trigger an inflammatory response.^{25, 26} The processes associated with lymphangitis and elephantiasis can take years to develop, and are not commonly seen in children. How living worms and microfilariae suppress the host inflammatory response is being explored. It has been noted that microfilariae produce prostaglandin E₂, a modulatory agent for leukocytes, and adult worms secrete anti-mitotic and immunosuppressive substances.²⁷ Adult worms can also influence host immune responses by releasing small RNA-containing exosome-like vesicles that influence host immune-cell gene expression.²⁸

When dead and dying adult worms relinquish control of the host's defense mechanisms, a series of inflammatory reactions result, causing alterations of the walls of the lymphatics. After an intense lymphocytic infiltration, the vessel's lumen eventually closes, and the remnants of the adult worms calcify. The blockage of lymphatic circulation continues in heavily infected individuals until most major lymph channels are occluded, causing lymphedema in the affected body region below the blocked vessels. In addition, hypertrophy of smooth muscle tissue occurs in the area immediately surrounding the site of involvement. When treated with agents that kill adult filarial worms, individuals can develop complications, such as developing new hydroceles.²⁹ The process of lymphatic blockage is protracted and results from repeated infections. Consequently, individuals who become infected by visiting endemic areas for short periods usually do not develop lymphedema.

Not all patients with chronic exposure of infective larvae of *W. bancrofti* develop overt clinical disease. It is unclear why, despite relatively equal levels of exposure, some infected residents remain largely asymptomatic but still harbor circulating microfilariae. In contrast, other individuals progress to advanced clinical disease comprised of lymphangitis and elephantiasis. Patients with advanced clinical disease often do not have evidence of circulating microfilariae.³⁰ Differences in host cytokine patterns have been noted among these different groups of patients. It has been suggested that different populations are prone to either Th2 or Th1 biases in their cellular inflammatory responses.^{25, 31, 32} Growing evidence suggests that host genetic variability accounts for the different clinical manifestations seen among patients with similar exposures.^{33, 34} The relationship between host and parasite is

complex, and there is often an equally complex pathologic sequence of events leading to lymphangitis, lymphedema, and elephantiasis. While much inflammation occurs once adults have died, there is evidence from ultrasound studies conducted in LF-endemic areas that the living adult filarial worms induce important pathologic changes, including lymphatic dilatation, which may lead to subsequent chronic lymphatic changes. Secondary bacterial and fungal infections contribute significantly to the chronic pathology of elephantiasis and are significant complications for patients with full-blown elephantiasis. Adult *W. bancrofti* harbor bacterial symbionts of the genus *Wolbachia*, and depend on them for survival. Antibiotics that target *Wolbachia* adversely affect the biology of the worm. *Wolbachia* contain endotoxin-like molecules, and evidence suggests that these molecules may contribute to the inflammatory responses against dead and dying worms. *Wolbachia* plays a major role in the pathogenesis of filarial disease. Some of the progression of clinical disease appears to be due to immune responses triggered by the *Wolbachia* endosymbiont.³⁵⁻³⁸

Clinical Disease

A spectrum of clinical manifestations results from *W. bancrofti* or *B. malayi* infections, ranging from asymptomatic infection to advanced elephantiasis and was comprehensively reviewed by Medeiros et al in 2021.³⁹

Asymptomatic Infection (Lymphatic Dilatation)

Most residents living in an endemic area do not manifest strong inflammatory responses to their filarial parasite load. They are asymptomatic even though they have circulating microfilariae. Some of these so-called asymptomatic patients have been observed to exhibit subtle pathology when examined more closely by ultrasound or radionuclide

studies.⁴⁰ The central event in the pathogenesis of more advanced disease may begin at this stage when dilatation of the lymphatic vessels begins to occur. Dilatation initiates a subsequent series of events that results in the chronic clinical manifestations of LF, including lymphedema and hydrocele.⁴¹ Sometimes, the dilated vessels rupture to produce chyluria and chylocele.

Acute Lymphadenitis and Filarial Fevers

Death of the adult worm leads to the progression of disease by producing an acute inflammatory response that is manifested as acute lymphadenitis. In endemic areas, this occurs frequently during the patient's adolescence and manifests with fevers and painful swellings over the lymph nodes.⁴² This typically occurs in the inguinal area. Episodes of painful swellings can last up to a week and commonly recur. Secondary bacterial infections may exacerbate acute filarial lymphadenitis.



Figure 22.5. Patient suffering from long-term infection with *W. bancrofti*. Most adult worms have died and calcified, blocking all lymphatic drainage from the groin. The result is elephantiasis of the leg.

Individuals who have traveled to and spent several months in endemic areas can also develop acute lymphadenitis, but the pathogenesis of this process occurs by a poorly understood process. This phenomenon was described in the 1940s among American troops returning from combat in the Pacific during World War II.⁴³

Elephantiasis

A subset of patients with acute lymphangitis and filarial fevers will go on to develop lymphedema of the arms, legs, breasts, and genitalia, leading to elephantiasis (Fig. 22.5). During these inflammatory processes, the skin becomes doughy. It exhibits some degree of pitting, though it is rather firm. As the inflammatory reaction continues, the area becomes firmer, and pitting disappears. There is substantial spread of the inflammation into the subcutaneous tissue and consequent loss of elasticity of the overlying skin. Characteristically, and in contrast to cellulitis caused by some bacteria, filarial cellulitis shows no demarcation line between the affected and the healthy skin. In bancroftian filariasis, the legs are more likely to be involved than the upper extremities, and the lower portions of the legs are more involved than the upper ones. The scrotum is frequently affected in the form of hydroceles and may become gigantic, weighing up to 10 kg; much larger scrotums have been described in rare cases.

Tropical Pulmonary Eosinophilia

Tropical pulmonary eosinophilia (TPE) develops in some individuals with filarial infections. This syndrome, which occurs frequently in southern India, particularly in young adult men, is characterized by high levels of serum IgE, nocturnal asthma with interstitial infiltrates on chest radiographs, fatigue, weight loss, and circulating eosinophilia.⁴⁴ Left untreated, TPE can progress to chronic restrictive lung disease. The pathogenesis of this syndrome is related to local

immune responses to microfilariae in the pulmonary vasculature and results in eosinophil accumulation in the lung with the release of cytotoxic eosinophil products (e.g., major basic protein and eosinophil cationic protein).⁴⁴

Diagnosis (see Clinical Appendix)

LF should be suspected in an individual who resides in an endemic region, is beyond the first decade of life, and has lymphedema in the extremities or genitalia. Definitive diagnosis has traditionally depended upon microscopically observing the characteristic microfilariae in the blood (Figs. 22.2, 22.3, C.46–C.53). Occasionally, infection is so heavy that microfilariae can be observed on a thin blood smear stained with Giemsa. In lighter infections, methods include filtering blood onto a 0.45 μm pore-sized nucleopore filter and then staining it with Giemsa solution. In the case of very light infection, 1 ml of blood is preserved in 9 ml of 1% formalin and then concentrated by centrifugation (Knott's test; Appendix B). The pellet contains red blood cell ghosts and microfilariae. Stained smears of the pellet are then examined microscopically. Because of the nocturnal periodicity of some strains, it is best to draw blood during the customary hours of sleep (usually 22:00–02:00 hours).

Antigen tests that detect circulating *W. bancrofti* antigens have been developed, which are more sensitive than microscopy, and can be used for daytime testing. These tests can give a quantitative result correlating with adult worm burden.^{45–47} They are available as an ELISA test or an immunochromatographic card.⁴⁸ The card-based assay, which recognizes the adult worm's 200 kDa antigen, has a sensitivity of 96–100% and a specificity approaching 100%.⁴⁹ The ELISA also has a sensitivity approaching 100% in microfilaremic patients. For both assays, the circulating

filarial antigen remains diurnally constant so that blood for diagnosis can be collected during the day. PCR-based tests have been developed, but while used to monitor filarial infection in mosquitoes, they are currently not routinely used in clinical practice.⁵⁰

Serological tests that detect levels of human IgG directed against the worms that cause LF have been developed and are in use clinically. These serve as rapid tests that can aid in diagnosing this disease. They detect exposure rather than confirm current infection.^{51–53}

Increasingly, ultrasound has provided an important noninvasive modality for monitoring the efficacy of antifilarial drugs.³⁵ Ultrasound examination of the lymphatic vessels of the spermatic cord of infected men results in a distinctive sign, known as the “filarial dance sign” reflective of nests of live worms in the lymphatics.⁵⁴ Adult worm death following treatment with DEC can be subsequently followed.

Treatment (see Clinical Appendix)

It is recommended that all patients be treated because even patients with so-called asymptomatic infection may have abnormal lymphatics, and there is increasing evidence that early treatment may prevent subsequent lymphatic damage and may reverse early lymphatic dysfunction.⁵⁵ It is critical that, before treatment, co-infection with *Loa loa* and a high microfilarial load is ruled out due to the risk of severe adverse pathological events if an ivermectin containing regimen is given to such patients.⁵⁶ For the treatment of mono-infected patients, DEC has partial macrofilaricidal (adult worm) and microfilaricidal properties and is the treatment of choice for such patients. In many regions, it is given in a dose of 6 mg/kg/day for 12 consecutive days for a total of 72 mg/kg body weight.^{35,57} For *W. bancrofti* infections, this results in at least a

90% decrease in microfilaremia within one month. DEC decreases the incidence of filarial lymphangitis and in some cases, reverses existing lymphatic damage. The addition of doxycycline for 4–6 weeks may be beneficial based on evidence demonstrating macrofilaricidal activity and reduced pathology due to eliminating the endosymbiont bacteria *Wolbachia* from the worm gut.^{58–60} However, the length of the doxycycline therapy needed is impractical under field conditions and contraindicated in pregnant women and children <8 years of age for prolonged treatment hence new drugs are needed and analogues of Tylosin A have demonstrated promising activity in animal models.⁶¹

In men, the efficacy of treatment can be monitored by serial ultrasound examinations (see above), and by serial blood sampling.³⁵ Since DEC is only partially effective against the adult worm, repeat treatments are often required. This is often done every 6–12 months.³⁵ Some data suggests that single dose treatment with 6 mg/kg of DEC has comparable macrofilaricidal and long-term microfilaricidal activity. Some clinicians have suggested that single-dose treatment can be repeated every 6–12 months.³⁵ DEC is associated with fever (probably resulting from the disintegration of a few adult worms), occasional nausea and vomiting, and transient skin rashes. Ivermectin, a drug effective for the therapy of onchocerciasis, also kills microfilariae of *W. bancrofti*, but it has no effect against adult worms. Albendazole, a drug that also does not kill adult filarial worms, has been used in mass drug campaigns, reducing microfilarial blood levels and decreasing transmission.⁶²

Aside from the use of antihelminthic drugs, there are several treatment modalities that help to improve the chronic sequelae of LF, including lymphedema and elephantiasis. Both conditions, when they occur in the leg,

are reversible with a hygienic regimen that includes prevention of secondary bacterial infections by prompt antibiotic treatment of acute bacterial attacks, aggressive treatment of skin lesions, including those caused by *Candida* and other fungi, and physiotherapy.³⁵ Treatment of secondary bacterial infections has been identified as a critical treatment modality for lymphedema and elephantiasis. Hydrocele drainage without corrective surgery, while providing relief, is often associated with reaccumulating fluid.⁶³ For certain affected areas (e.g., the scrotum), corrective surgical interventions may be required.⁶⁴ Surgical techniques have been developed that can, in most cases, alleviate hydroceles and greatly improve the morbidity associated with filarial infections.⁶⁵

Prevention and Control

Patent microfilaremia is first detected in children 5–10 years old who live in endemic regions.⁷ Transplacental immunity and breastfeeding may limit the intensity of infection in younger individuals. The prevalence of microscopically confirmed infection gradually increases up to 30–40 years old.

Determining the frequency of occurrence of L3 larvae in local mosquito vector populations is the most important strategy in estimating the community prevalence of filariasis.⁶⁶ Prevention involves controlling mosquito vectors, which has had modest success because mosquitoes develop insecticide resistance.⁶⁷ Insecticide-treated bed nets have effectively reduced transmission in areas where anopheline mosquitos transmit the disease.⁶⁸ Hyper-urbanization of vast areas of tropical Asia has resulted in a concomitant rise in the prevalence of both *W. bancrofti* and *B. malayi* transmitted by mosquitoes that breed in peridomestic habitats.

In 1997, the World Health Assembly passed

a resolution calling on its member states to undertake a global elimination program for LF. The major strategy for LF elimination is based on two principles: 1) to interrupt transmission of infection and 2) to alleviate and prevent the suffering and disability caused by LF. Reducing the levels of microfilariae in the blood for a sustainable period is essential to interrupt transmission. Mass drug administration (MDA) is a community intervention that controls the spread of lymphatic filariasis infection through WHO-recommended regimens of different combinations of albendazole, diethylcarbamazine and ivermectin. The regimen will be determined by co-endemicity of loaiasis and onchocerciasis and whether the MDA program is advanced or just commencing. A three-drug regimen containing ivermectin, DEC and albendazole is recommended in WHO Guidelines for rapid anti-microfilaria activity in geographies initiating MDA where *Loa loa* and onchocerciasis are not present.⁶⁹ In many parts of Sub-Saharan Africa and Yemen where there is epidemiological overlap with onchocerciasis, the toxicities caused by DEC in people with these conditions necessitate substituting ivermectin (200 mcg/kg). Such populations would receive either ivermectin or albendazole.^{68,70} Ivermectin cannot be used in geographic areas known to be endemic for *Loa loa*. Multiple years of annual treatments are recommended with the number dependent on the regimen utilized and the results of on-going studies in progress to inform guidelines.⁷¹ The number of serious adverse events from LF control mass chemotherapy has been remarkably low. In some areas, a treatment regimen comprised of daily DEC-fortified salt is used. A Global Program to Eliminate LF in collaboration with the World Health Organization is leading these efforts.⁷²

The major strategy to alleviate suffering and decrease the disability caused by LF has been to decrease secondary bacterial and fungal

infections of the affected limbs and genitals. This includes meticulous local hygiene, judicious use of antibiotics, physiotherapy, and health education.

Currently, no effective vaccines are available to control LF at the community level, but research efforts continue toward their development.⁷³

References

- Knopp, S., et al., Nematode infections: filariases. *Infect Dis Clin North Am* **2012**, 26 (2), 359-81.
- Nelson, G. S., Current concepts in parasitology. Filariasis. *The New England journal of medicine* **1979**, 300 (20), 1136-9.
- Global programme to eliminate lymphatic filariasis: progress report for 2012. *Wkly Epidemiol Rec* **2013**, 88 (37), 389-99.
- Global programme to eliminate lymphatic filariasis. *Wkly Epidemiol Rec* **2010**, 85 (38), 365-72.
- Taylor, M. J., et al., Lymphatic filariasis and onchocerciasis. *Lancet* **2010**, 376 (9747), 1175-85.
- Organization, W. H., Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva. Licence: CC BY-NC-SA 3.0 IGO **2020**.
- Nanduri, J.; Kazura, J. W., Clinical and laboratory aspects of filariasis. *Clinical microbiology reviews* **1989**, 2 (1), 39-50.
- Baird, J. K., et al., North American brugian filariasis: report of nine infections of humans. *The American journal of tropical medicine and hygiene* **1986**, 35 (6), 1205-9.
- Molyneux, D. H., et al., Mass drug treatment for lymphatic filariasis and onchocerciasis. *Trends in parasitology* **2003**, 19 (11), 516-22.
- Molyneux, D. H.; Zagaria, N., Lymphatic filariasis elimination: progress in global programme development. *Ann Trop Med Parasitol* 96 Suppl 2 **2003**, S15-40.
- Global Burden of Disease Study, C., Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **2015**, 386 (9995), 743-800.
- Hotez, P. J., et al., Combating tropical infectious diseases: report of the Disease Control Priorities in Developing Countries Project. *Clin Inf Dis* **2004**, 38 (6), 871-8.
- Demarquay, M., Note sur une tumeur des bourses contenant un liquide laiteux (galactocoele de Vidal) et refermant des petits etres vermiformes que l'on peut considerer comme des helminths hematoides a l'etat d'embryon. *Gaz Med Pans.* **1863**, 18, 665-667.
- Wucherer, O. E., Sobre a molestia vulgarmente denominada oppilacao ou cancao. *Gaz. Med. Bahia* **1866**, 1, 27-29, 39-41, 52-54, 63-64.
- Lewis, T. R., On a haematozoon inhabiting human blood, its relation to chyluria and other diseases. *8th Annual Report of the Sanitary Commission Government of India. Sanitary Commission Government of India, Calcutta.* **1872**, 241-266.
- Cox, F. E., History of human parasitology. *Clin Microbiol Rev* **2002**, 15 (4), 595-612.
- Cobbold, T. S., Discovery of the adult representative of microscopic filariae. *Lancet* **1877**, 2 70-71.
- Lewis, T., *Filaria sanguinis hominis* (mature form), found in a bloodclot in naevoid elephantiasis of the scrotum. *Lancet* **1877**, 2, 453-455.
- Manson, P., Further Observations on *Filaria sanguinis hominis*. *Medical Reports, China Imperial Maritime Customs Shanghai no 14 pp* **1878**, 1-26.
- Gwadz, R. W.; Chernin, E., Oral transmission of *Brugia pahangi* to Jirds (*Meriones unguiculatus*). *Nature* **1972**, 239 (5374), 524-5.
- Hawking, F.; Marques, R. J., Control of Bancroftian filariasis by cooking salt medicated with diethylcarbamazine. *Bull World Health Organ* **1967**, 37 (3), 405-14.
- Hawking, F., A review of progress in the chemotherapy and control of filariasis since 1955. *Bull World Health Organ* **1962**, 27, 555-68.
- Hawking, F., et al., The periodicity of microfilariae. XI. The effect of body temperature and other

- stimuli upon the cycles of *Wuchereria bancrofti*, *Brugia malayi*, *B. ceylonensis* and *Dirofilaria repens*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1966**, 60 (4), 497-513.
24. Carme, B.; Laigret, J., Longevity of *Wuchereria bancrofti* var. *pacifica* and mosquito infection acquired from a patient with low level parasitemia. *The American journal of tropical medicine and hygiene* **1979**, 28 (1), 53-5.
 25. Ottesen, E. A., The Wellcome Trust Lecture. Infection and disease in lymphatic filariasis: an immunological perspective. *Parasitology* **1992**, 104 Suppl, S71-9.
 26. King, C. L., et al., Immunologic tolerance in lymphatic filariasis. Diminished parasite-specific T and B lymphocyte precursor frequency in the microfilaremic state. *J Clin Invest* **1992**, 89 (5), 1403-10.
 27. Liu, L. X., et al., Release of prostaglandin E2 by microfilariae of *Wuchereria bancrofti* and *Brugia malayi*. *The American journal of tropical medicine and hygiene* **1992**, 46 (5), 520-3.
 28. Zamanian, M., et al., Release of small RNA-containing exosome-like vesicles from the human filarial parasite *Brugia malayi*. *PLoS Negl Trop Dis* **2015**, 9 (9), e0004069.
 29. Hussein, O., et al., Duplex Doppler sonographic assessment of the effects of diethylcarbamazine and albendazole therapy on adult filarial worms and adjacent host tissues in Bancroftian filariasis. *Am J Trop Med Hyg* **2004**, 71 (4), 471-7.
 30. Dissanayake, S., In *Wuchereria bancrofti* filariasis, asymptomatic microfilaraemia does not progress to amicrofilaraemic lymphatic disease. *Int J Epidemiol* **2001**, 30 (2), 394-9.
 31. Almeida, A. B., et al., The presence or absence of active infection, not clinical status, is most closely associated with cytokine responses in lymphatic filariasis. *Dis* **1453** **1996**, 173.
 32. Piessens, W. F., et al., Immune responses in human infections with *Brugia malayi*: specific cellular unresponsiveness to filarial antigens. *The Journal of clinical investigation* **1980**, 65 (1), 172-9.
 33. Lammie, P. J., et al., The pathogenesis of filarial lymphedema: is it the worm or is it the host? *Ann N Y Acad Sci* **2002**, 979, 131-42; discussion 188-96.
 34. Choi, E. H., et al., Genetic variation in immune function and susceptibility to human filariasis. *Expert Rev Mol Diagn* **2003**, 3 (3), 367-74.
 35. Taylor, M. J., A new insight into the pathogenesis of filarial disease. *Current molecular medicine* **2002**, 2 (3), 299-302.
 36. Taylor, M. J., *Wolbachia* in the inflammatory pathogenesis of human filariasis. *Ann N Y Acad Sci* **2003**, 990, 444-9.
 37. Tamarozzi, F., et al., Human filarial *Wolbachia* lipopeptide directly activates human neutrophils *in vitro*. *Parasite Immunol* **2014**, 36 (10), 494-502.
 38. Turner, J. D., et al., *Wolbachia* lipoprotein stimulates innate and adaptive immunity through Toll-like receptors 2 and 6 to induce disease manifestations of filariasis. *J Biol Chem* **2009**, 284 (33), 22364-78.
 39. Medeiros, Z. M., et al., Lymphatic Filariasis: A Systematic Review on Morbidity and Its Repercussions in Countries in the Americas. *Int J Environ Res Public Health* **2021**, 19 (1).
 40. Freedman, D. O., et al., Lymphoscintigraphic analysis of lymphatic abnormalities in symptomatic and asymptomatic human filariasis. *The Journal of infectious diseases* **1994**, 170 (4), 927-33.
 41. Addiss, D. G., et al., Treatment of lymphatic filariasis. Vol. 2000, p 151-199.
 42. Pani, S. P., et al., Episodic adenolymphangitis and lymphoedema in patients with bancroftian filariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1995**, 89 (1), 72-4.
 43. Huntington, R. W., et al., Filariasis Among American Troops in a South Pacific Island Group. *Yale J Biol Med* **1944**, 16 (5), 529-538 1.
 44. Ottesen, E. A.; Nutman, T. B., Tropical pulmonary eosinophilia. *Annual review of medicine* **1992**, 43, 417-24.
 45. El-Moamly, A. A., et al., Using the AD12-ICT rapid-format test to detect *Wuchereria bancrofti* circulating antigens in comparison to Og4C3-ELISA and nucleopore membrane filtration and microscopy techniques. *Parasitol Res* **2012**, 111 (3), 1379-83.
 46. Rocha, A., et al., Comparison of tests for the detection of circulating filarial antigen (Og4C3-

- ELISA and AD12-ICT) and ultrasound in diagnosis of lymphatic filariasis in individuals with microfilariae. *Mem Inst Oswaldo Cruz* **2009**, 104 (4), 621-5.
47. Wattal, S., et al., Evaluation of Og4C3 antigen ELISA as a tool for detection of bancroftian filariasis under lymphatic filariasis elimination programme. *J Commun Dis* **2007**, 39 (2), 75-84.
 48. Cunningham, J., et al., A global comparative evaluation of commercial immunochromatographic rapid diagnostic tests for visceral leishmaniasis. *Clin Infect Dis* **2012**, 55 (10), 1312-9.
 49. Well, G. J., et al., A monoclonal antibody-based enzyme immunoassay for detecting parasite antigenemia in Bancroftian filariasis. *Dis* **1987**, 156, 350-355.
 50. Wijegunawardana, A. D., et al., Evaluation of PCR-ELISA as a tool for monitoring transmission of *Wuchereria bancrofti* in District of Gampaha, Sri Lanka. *Asian Pac J Trop Biomed* **2013**, 3 (5), 381-7.
 51. Steel, C., et al., Rapid *Wuchereria bancrofti*-specific antigen Wb123-based IgG4 immunoassays as tools for surveillance following mass drug administration programs on lymphatic filariasis. *Clin Vaccine Immunol* **2013**, 20 (8), 1155-61.
 52. Steel, C., et al., Antibody to the filarial antigen Wb123 reflects reduced transmission and decreased exposure in children born following single mass drug administration (MDA). *PLoS Negl Trop Dis* **2012**, 6 (12), e1940.
 53. Kubofcik, J., et al., Identification of Wb123 as an early and specific marker of *Wuchereria bancrofti* infection. *PLoS Negl Trop Dis* **2012**, 6 (12), e1930.
 54. Mand, S., et al., Animated documentation of the filaria dance sign (FDS) in bancroftian filariasis. *Filaria J* **2003**, 2 (1), 3.
 55. Moore, T. A., et al., Diethylcarbamazine-induced reversal of early lymphatic dysfunction in a patient with bancroftian filariasis: assessment with use of lymphoscintigraphy. *Clin Infect Dis* **1996**, 23 (5), 1007-11.
 56. Bhalla, D., et al., Neurological manifestations of filarial infections. *Handb Clin Neurol* **2013**, 114, 235-42.
 57. Kazura, J. W., et al., Filariasis. *Tropical Infectious Diseases, Principles, Pathogens, & Practice* **1999**, 2, 852-860.
 58. Mand, S., et al., Doxycycline improves filarial lymphedema independent of active filarial infection: a randomized controlled trial. *Clin Infect Dis* **2012**, 55 (5), 621-30.
 59. Mand, S., et al., Macrolaricidal activity and amelioration of lymphatic pathology in bancroftian filariasis after 3 weeks of doxycycline followed by single-dose diethylcarbamazine. *Am J Trop Med Hyg* **2009**, 81 (4), 702-11.
 60. Taylor, M. J.; Hoerauf, A., A new approach to the treatment of filariasis. *Current opinion in infectious diseases* **2001**, 14 (6), 727-31.
 61. Taylor, M. J., et al., Preclinical development of an oral anti-Wolbachia macrolide drug for the treatment of lymphatic filariasis and onchocerciasis. *Sci Transl Med* **2019**, 11 (483).
 62. Demebele, B., et al., Use of high-dose, twice-yearly albendazole and ivermectin to suppress *Wuchereria bancrofti* microfilarial levels. *Clin Infect Dis* **2010**, 51 (11), 1229-35.
 63. Freeman, C. D., et al., Metronidazole. A therapeutic review and update. *Drugs* **1997**, 54 (5), 679-708.
 64. Capuano, G. P.; Capuano, C., Surgical management of morbidity due to lymphatic filariasis: the usefulness of a standardized international clinical classification of hydroceles. *Trop Biomed* **2012**, 29 (1), 24-38.
 65. Addiss, D. G.; Brady, M. A., Morbidity management in the Global Programme to Eliminate Lymphatic Filariasis: a review of the scientific literature. *Filaria J* **2007**, 6, 2.
 66. Piessens, W. F.; Partono, F., Host-vector-parasite relationships in human filariasis. *Semin Infect Dis* **1980**, 3 131-152.
 67. Davis, E. L., et al., Modelling the Impact of Vector Control on Lymphatic Filariasis Programs: Current Approaches and Limitations. *Clin Infect Dis* **2021**, 72 (Suppl 3), S152-S157.
 68. Global Programme to Eliminate Lymphatic Filariasis. *Wkly Epidemiol Rec* **2006**, 81 (22), 221-32.
 69. WHO, Lymphatic filariasis <https://www.who.int/news-room/fact-sheets/detail/lymphatic->

[filariasis#:~:text=WHO%20recommends%20the%20following%20MDA,in%20countries%20without%20onchocerciasis%3B%20and.](#)

70. Sabesan, S., Albendazole for mass drug administration to eliminate lymphatic filariasis. *Lancet Infect Dis* **2006**, *6* (11), 684-5.
71. Pfarr, K. M., et al., The pipeline for drugs for control and elimination of neglected tropical diseases: 2. Oral anti-infective drugs and drug combinations for off-label use. *Parasites & vectors* **2023**, *16* (1), 394.
72. WHO, Alternative mass drug administration regimens to eliminate lymphatic filariasis. Licence: CC BY-NC-SA 3.0 IGO. . **2017**.
73. Kalyanasundaram, R., et al., Advances in Vaccine Development for Human Lymphatic Filariasis. *Trends Parasitol* **2020**, *36* (2), 195-205.

23. *Onchocerca volvulus*

(Leuckart 1893)

Pronunciation: \äŋ-kə-'sər-kə\\väl-vyə-ləs\

Introduction

Onchocerca volvulus (\ONG-ko-ser-kah\\VOL-view-lus\) is a vector-borne, filarial nematode parasite and the causative agent of river blindness. The adult worm lives in the subcutaneous tissues. Its offspring, microfilariae, migrate and induce injury to various anatomical sites contiguous with that tissue. No reservoir hosts exist for *O. volvulus*.¹ The black fly, *Simulium* (\seh-MYOO-LEE-um\\,\\si-'myü-lē-əm\) spp., is the vector. *O. volvulus* occurs mostly in West and Central Africa. Onchocerciasis used to be endemic in Central and South America but has been eliminated in 4 countries and only now remains among indigenous populations living on the Brazil-Venezuela border.²

Onchocerciasis was the major cause of blindness throughout Sub-Saharan Africa, often affecting more than 50% of towns and villages in endemic areas.³ River blindness is

now the second-leading infectious cause of blindness in the world (trachoma is first), with approximately half a million people going blind due to *O. volvulus*.⁴ The disease also causes disfiguring dermatitis that at one time was second only to polio as a cause of long-term disability in endemic areas. *O. volvulus* was once so prevalent that people could not live in many places along riverbanks.⁵

Current estimates indicate that approximately 25 million people remain infected worldwide, with 99% or more living in Sub-Saharan Africa. The African Programme for Onchocerciasis Control (APOC, 1995-2015), a shared partnership led by the World Bank, the World Health Organization, the United Nations Development Programme, and the Food and Agriculture Organization of the United Nations, coordinated the annual mass treatment with ivermectin (a gift from Merck Pharmaceuticals) in 19 countries and had a highly cost-effective impact on controlling this disease.⁶ APOC built on the initial success of the Onchocerciasis Control Program (OCP, 1974-2002) and worked to extend its reach to all endemic countries in Central and East Africa.⁶⁻⁸ The Mectizan donation program is managed by the Taskforce for Global Health with technical support provided since 2015 from the Expanded Special Project for the Elimination of Neglected Tropical Diseases (ESPEN).^{9, 10} In South America, thanks in large part to the efforts of the Onchocerciasis Elimination Program for the Americas (OEPA), active transmission is limited to two foci in Venezuela and Brazil.¹¹ The progress toward the goal of elimination and guidelines for additional steps are outlined in the 2020 WHO NTD roadmap.¹²⁻¹⁵ WHO issued guidelines in 2016 for when to stop mass onchocerciasis treatment programs and for formal certification of disease elimination in a given geographic region.¹⁶

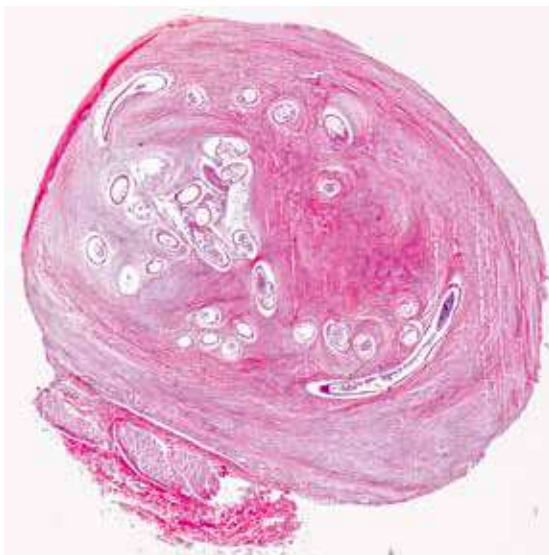
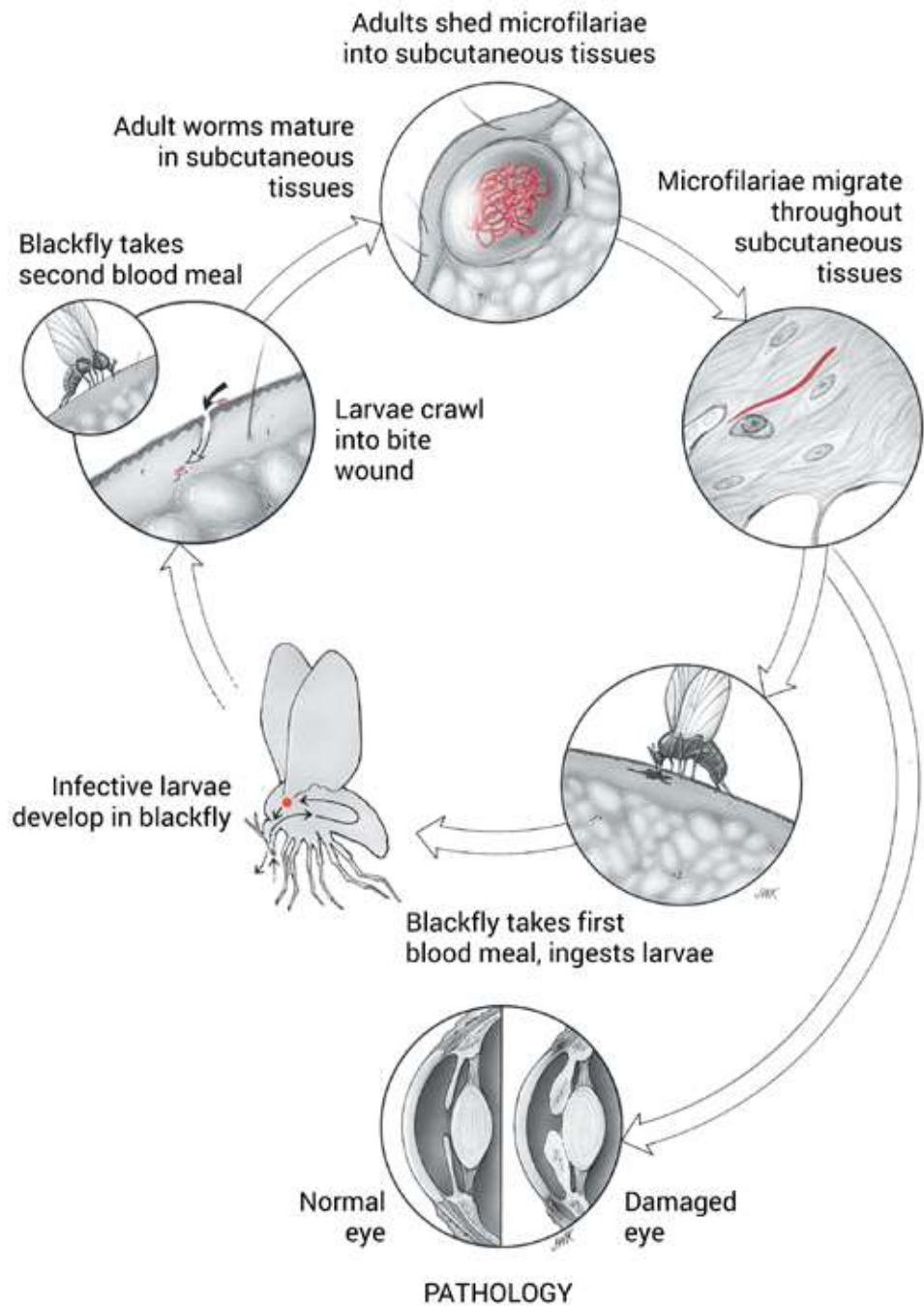


Figure 23.1. Cross section of nodule (onchocercoma) induced by *Onchocerca volvulus*. Numerous sections of adult worms are seen. 2.5 cm diameter.

Onchocerca volvulus



Historical Information

In 1874, an Irish naval surgeon, John O'Neill, discovered microfilaria in skin snips of individuals in Ghana.¹⁷ Credit for the first description of *O. volvulus* is given to Karl Georg Friedrich Rudolf Leuckart, who recounted his discovery of the parasite to Patrick Manson, who, in turn, published the full description in 1893, giving Leuckart full credit.¹⁸ Onchocerciasis in Latin America was not reported until 1917 when Rodolfo Robles found ocular disease associated with the presence of nodules on the forehead of a small boy.¹⁹ He dissected the nodule and found that it contained the adult worms. Later, Robles described the worm's anatomy, the disease's pathology, and the infection's epidemiology. Moreover, he suspected that the black fly was the vector, later proved by Donald Blacklock in 1927.²⁰

Life Cycle

Adult females measure 20–80 cm by 0.3 cm, while males measure 3–5 cm in length. Both sexes lie entwined about each other, locating to subcutaneous fibrous nodules called onchocercomas (Fig. 23.1), which vary in size depending on the number of adult worms in them. The male worms can migrate between nodules to fertilize females, likely in response to chemoattractants released by female worms.²¹ Some nodules are so small that they cannot be detected.²² Microfilariae are produced within the nodules, leaving these sites to migrate throughout the subcutaneous tissues (Figs. 23.2, 23.3).

Only female black flies (Fig. 38.5) acquire the larvae while taking a blood meal; males do not feed on blood. The black fly injects anticoagulants, creating a blood meal in the subcutaneous tissue from which it ingests blood along with the microfilariae.²³ The immature worms penetrate the insect's

hemocoel and the muscle fibers of the flight wing bundles in the thorax. The larvae molt twice during a 6–8 day period of development. The now infective L3 larvae leave the muscles, enter the proboscis, and are deposited on the skin when the fly bites. Larvae enter the bite wound after the fly withdraws its biting mouthparts. The immature parasites invade the subcutaneous tissues with the aid of a protease and take up residence there.²⁴ Growth and molting of worms in the subcutaneous tissues induce the formation of the fibrous nodules and elicit an angiogenic response, producing a network of vessels, which presumably supply nutrients to the parasites and carry away metabolic wastes. The Nurse-cell-parasite complex of *Trichinella spiralis*^{17,18} induces a similar angiogenic response.²⁵ After completing their development, mating ensues.

There is a pre-patent period of 10–12 months after initial infection before the females begin producing microfilariae. Hundreds to thousands of microfilariae are shed during the female's life span of 8–10 years (about 700 microfilariae per day).

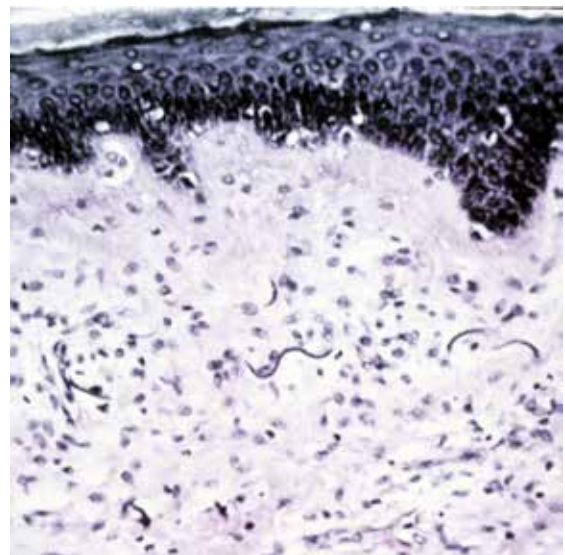


Figure 23.2. Section of skin with numerous microfilariae of *O. volvulus*.



Figure 23.3. Higher magnification of a microfilaria of *O. volvulus* in skin. 310 μm x 7 μm .

O. volvulus parasites contain microbial symbionts of the genus *Wolbachia*, as do other filaria, including *Wuchereria bancrofti*. These are rickettsia-like organisms that are found in the body wall, in oocytes, and in all embryonic stages, including microfilariae.²⁶ The *Wolbachia* are believed to be essential for nematode fertility and are transmitted transovarially to the next worm generation in a manner similar to mitochondria.²⁷ *Wolbachia* also contain endotoxin-like products that are proinflammatory. This has led to the hypothesis that these microbial symbionts contribute significantly to the skin and eye pathology of *O. volvulus*-infected patients.^{26, 27}

Cellular and Molecular Pathogenesis

Onchocerca larvae migrate through the tissues with macromolecules that promote tissue degradation, angiogenesis, and plasmin-mediated proteolysis.^{24, 28-30} *O. volvulus* has impressive immunomodulatory properties, which can bias host responses to a Th2-type pattern. By this mechanism, host-cell-mediated Th1-type immunity is suppressed, leading to impaired responses to purified protein derivative (PPD) skin testing for tuberculosis, tetanus, and other vaccinations, and increased susceptibility to intercurrent infections with lepromatous leprosy.³¹⁻³³ Down-regulation of the host immune response is thought to be induced by excretory-secretory products of *O. volvulus*, including omega-

class glutathione transferase 3 (OvGST).³⁴

The degree of pathogenesis varies directly with the intensity of infection and the degree of host responsiveness to dying adult worms and microfilariae and their secretions. The release of *Wolbachia*-derived antigens upon the death of the filariae is thought to play an essential role in triggering the destruction caused by the host's innate immune cells.³⁵

³⁶ Dead microfilariae induce inflammatory reactions that become more severe as the infection persists; this point is important when considering therapy. The lesions, primarily involving the skin and the eyes, occur due to cell-mediated immunity to parasite antigens. Individuals with the most vigorous cell-mediated immune responses develop the most severe manifestations.^{3, 30, 37} The magnitude of the immunopathologic response significantly influences the severity of clinical onchodermatitis.^{37, 38} Host mast cells play an important role in this phenomenon.³⁸

The major ocular lesions occur in the cornea to produce keratitis.³⁹ The keratitis leads to an accumulation of punctate opacities in the cornea arising from immune-mediated damage resulting from the reaction to microfilariae in the eye. This is a Th2-dependent process that relies heavily on host interleukin-4 and neutrophil recruitment to the corneal stroma.^{39, 40} While corneal pathology appears to be in response to both *Wolbachia* and microfilarial antigens, the skin reactions appear to be mainly, if not exclusively, due to filarial antigens.⁴¹ In the skin, this leads to pruritus and angioedema.

The pathogenesis of onchocerciasis depends on the number of worms infecting an individual and certain host factors. Polymorphisms in the *FcyRIIIa* gene that impact binding affinities of immunoglobulin subclasses have been correlated with clinical outcomes.³⁷ Genome-wide association studies have

detected genetic variants associated with disease.⁴² Hyperreactive responses that trigger the skin manifestation known as *sowda* have been linked to variants of the interleukin-13 gene that lead to elevated levels of IgE and overly exuberant immune responses.⁴³

Subcutaneous nodules, the other hallmark of clinical onchocerciasis, vary from barely discernable to approximately 5 cm in diameter. Nodules develop over 18 months depending on the number of adult worms in each. The number of nodules also varies, from occasional to several hundred, occupying large areas of subcutaneous tissue. In the latter instance, black flies biting such individuals may expire due to the overwhelming nature of the infection in their flight wing muscles. Those areas where peripheral lymphatics converge (e.g., occiput, suboccipital areas, intercostal spaces, axilla, and iliac crests) have the highest predilection for nodules. The body regions most affected differ according to geographic locales. In Africa, the nodules predominate in the lower part of the body, whereas in Central America, they tend to be found more often in the upper portions. This difference is related to the biting habits of the vector insects and the clothing styles worn by each endemic area's inhabitants.

Clinical Disease

Clinical onchocerciasis includes dermatitis, eye lesions, onchocercomas, and systemic disease.

Onchodermatitis

The major skin manifestations of onchocerciasis are papular (acute and chronic), lichenified (*sowda*), atrophy (hanging groin), and depigmentation (leopard skin).⁴⁴⁻⁴⁶ Mild infection (less than five nodules per infected individual) is usually asymptomatic. In contrast, moderate to severe infection (ten or more nodules, with many in the head and

neck region) produce correspondingly more serious and more numerous symptoms. In acute papular onchodermatitis there are pruritic papules, vesicles and pustules mainly on the buttocks and shoulders.⁴⁷ In chronic papular onchocerciasis the papules are slightly larger and symmetrically distributed in these same areas but may also involve the waist and display hyperpigmentation. Lichenified skin manifestations tend to be geographically limited to areas such as the Sudan and Yemen and are named for the dark color of the plaques from the Arabic word (*sowda*) for dark.^{48, 49} There have been rare descriptions of this occurring outside these geographical confines including cases reported in the New World.^{50, 51}

Skin atrophy may develop from loss of skin elasticity and the skin may take on the appearance of tissue paper in areas around the waist, buttocks, and upper legs.^{44, 45} Hanging groin can be associated with intense pruritus and the development of hernias.^{52, 53} Leopard skin is a manifestation with dermal hypopigmentation that has gained some attention as a low-tech means of rapidly estimating prevalence of onchocerciasis, but tends to only develop in older individuals.^{5, 54, 55} Occasionally, the pruritus of onchodermatitis is intense and disabling. It is alleged that occasional suicides result from the extreme discomfort associated with it.³ A reported manifestation in infected Central American children involved facial lesions that were reddish in color, described as *erysipelas de la costa*.⁵⁶

Lymphadenopathy

Lymph node involvement in Africa is usually found in the inguinal and femoral nodes, whereas in the American tropics it is in the head and neck.³

Ocular Lesions

Ocular manifestations of onchocerciasis include keratitis (punctate and sclerosing), iritis, uveitis, optic atrophy, optic neuritis, cataracts and chorioretinitis with the major cause of blindness being corneal blindness.⁵⁷ All parts of the eye are affected in chronic, long-term infections. Initially, there may be conjunctivitis, with irritation, lacrimation, and photophobia, a reaction analogous to the dermatitis in response to dead microfilariae. In general, the ocular manifestations appear to result from damage due to immune cells triggered by antigens from microfilaria and the Wolbachia bacteria endosymbiont. The cornea initially may display the punctate lesions of keratitis. The anterior chamber is also invaded, and microfilariae can be seen with a slit lamp, revealing motile or dead microfilariae in the conjunctiva.

A long-standing infection produces sclerosis and vascularization. Sclerosing keratitis of the cornea is the leading cause of blindness due to onchocerciasis, and develops over a 20–30-year period.⁵⁷ Onchocercal blindness peaks in those between 30–40 years of age, when individuals are most responsible for caring for their families. Little evidence suggests that sclerosing keratitis can be reversed sufficiently to restore vision once it has developed.⁵⁸

Finally, there may be iritis, iridocyclitis, and secondary glaucoma. Invasion of the posterior segment of the eye causes optic neuritis and papillitis; the choroid and the retina can also be involved. It remains unclear if this is due to a direct effect of the parasite or an immune response due to molecular mimicry.

Nodding Syndrome

A neurological disorder known as ‘nodding syndrome’ has been recognized among school-aged children and adolescents living in some onchocerciasis-endemic areas, espe-

cially in Uganda and in neighboring countries.⁵⁹ Although nodding syndrome has not been directly linked to onchocerciasis an epidemiological association has been noted.⁶⁰ Children with nodding syndrome exhibit periods of seizures and unresponsiveness comprised of multiple head nods, lack of responsiveness, and long-term mental disabilities. The etiology of nodding syndrome remains under investigation.⁶¹

Diagnosis (see Clinical Appendix)

Because of its highly focal distribution, a travel history or history of living in an endemic area is critical to entertain a clinical suspicion of onchocerciasis. Examining a skin snip can make a definitive diagnosis. This involves the bloodless removal of a 2–5 mm² piece of skin with a corneoscleral punch, a small beveled needle, or a disposable razor blade from an anatomical area thought to have the highest microfilaria levels. In Africa, the specimen should be obtained from the lower part of the body, and in Central America, from the upper part. The skin should be alcohol-cleansed, elevated with a needle, and cut with a scalpel blade. Next, a preferably bloodless piece should be placed in warm physiological saline and examined microscopically for motile microfilariae within 10 minutes. A representative sample of skin can be weighed, and the number of microfilariae per milligram of tissue calculated as an index of the intensity of infection. In addition, the piece of skin can be pressed against a dry microscope slide, and the impression stained with Giemsa solution and examined microscopically for microfilariae (Fig. 23.4). When performed, it is recommended that 6 snips be taken to increase sensitivity. Although skin snip microscopy has excellent specificity approaching 100%, it is only sensitive enough to make the diagnosis in less than half of cases, even with multiple skin snips.⁶² Histologic sections of a subcutane-

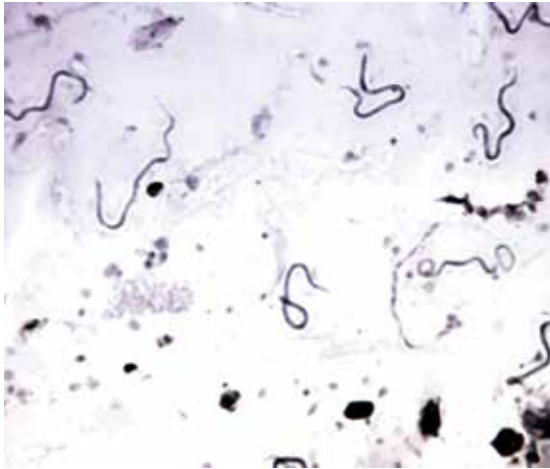


Figure 23.4. Impression smear of a skin snip from a patient heavily infected with *O. volvulus*. Microfilariae were visualized with Giemsa stain.

ous nodule (Fig. 23.2, 23.3) may also reveal microfilariae. The sensitivity of skin snips was improved by PCR amplification, but this approach still requires the collection of skin tissue specimens.^{63, 64}

Highly sensitive and specific rapid serological tests have been developed, but are not in wide use and cannot reliably distinguish between past versus current active infection in endemic areas.⁶⁵ Both urine and a tear antigen dipstick assay have been developed with high sensitivities, but as yet are not commercially available. The Mazzotti test is a provocative challenge test using a 50 mg dose of diethylcarbamazine citrate (DEC). Within 3 hours of treatment, patients with *O. volvulus* infection will develop pruritus. In heavily infected patients, the Mazzotti reaction can be severe and may exacerbate the ocular pathology in a patient. As an alternative, some physicians perform a type of patch test by applying DEC to a small region in order to elicit a local Mazzotti-like reaction.⁶⁶

Ultrasound has been used to visualize adult worms in nodules as well as to monitor their viability following the initiation of therapy.⁶⁷

Treatment (see Clinical Appendix)

Ivermectin is the drug of choice for onchocerciasis. Ivermectin inhibits the release of microfilariae from the female and can reduce microfilarial counts by up to 90% within one week.⁶⁸ A single oral dose of 150 mcg/kg administered every 6 months can slow or reverse the progression of ocular and cutaneous diseases.⁶⁹ The drug is available through the Mectizan® Donation Program established in 1988 by Merck & Co. Ivermectin does not kill the adult worms encased in a nodule. Therefore, repeat dosing is necessary to suppress the release of microfilariae for the adult worms' entire 10–17-year life span. In some patients, more frequent interval dosing is required to suppress pruritus. The major toxicity of ivermectin is generally not from the drug itself but rather from its ability to increase the antigen load from dead and dying parasites, leading to fever, angioedema, and pruritus. These symptoms usually occur within 24 hours of treatment. Moxidectin was approved in 2018 by the U.S. FDA for onchocerciasis in people >12 years of age. Further clinical work in younger age groups and pragmatic issues for field use will clarify the potential of this new medicine given its superior activity against the microfilaria of onchocerciasis.⁷⁰

In those patients with concurrent *Loa loa* infection, ivermectin can elicit severe reactions, including encephalopathy and consequently it is essential to evaluate the patients in areas endemic for *Loa loa* for co-infection.⁷¹ This point is especially critical in areas such as West and Central Africa, where there is epidemiologic overlap between the two helminth infections. In Latin America, the surgical removal of palpable subcutaneous nodules has led to successful resolution of the infection in some instances.

The possible role of *Wolbachia* endosymbionts in the inflammatory processes that caused eye and skin changes in *O. volvulus* infection, as well as their role in embryogenesis and parasite fertility, has led to the suggestion that antibiotics could have a therapeutic activity for patients with onchocerciasis.⁷² Prolonged administration of doxycycline (200 mg/day for 4–6 weeks) was shown to interrupt *O. volvulus* embryogenesis.²⁶

Prevention and Control

O. volvulus distribution follows that of the dipteran vectors. Black flies breed in fast-running water of mountainous streams in regions of Africa and South and Central America and have a fairly long flight range. Thus, onchocerciasis can be found several miles from the nearest endemic breeding site. Because much of the world's coffee is grown on mountainous hillsides, the prevalence of onchocerciasis among workers on coffee plantations is high. The OCP was launched in 1974, primarily emphasizing reducing larval black fly populations with DDT and other insecticides.⁷³ When ivermectin became widely available via the Mectizan donation program, the OCP focused on the use of this drug as an agent of mass chemotherapy.⁷⁴ Community-wide chemotherapy can interrupt the transmission of onchocerciasis.^{75, 76}

In Africa, efforts to control onchocerciasis were expanded by the APOC.⁷ Critical to the success of APOC up to 2015 and currently, is the Mectizan Donation Program, one of the first and largest public-private partnerships devoted to a neglected disease. This program was launched in 1987 when Roy

Vagelos, then CEO of Merck, made a historic announcement that his company would donate Mectizan® to anyone who needed it for as long as it was needed.^{7, 74} The Mectizan donation program as managed over 12 billion doses of the medicine for onchocerciasis and lymphatic filariasis by the end of 2023.⁹ One of the major innovations of APOC was the implementation of a novel system of community-directed treatment programs. In addition, the community-based health systems created by APOC provided a framework for additional pro-health interventions in low-income countries, including those that target other neglected diseases such as soil-transmitted helminth infections (STHs), schistosomiasis and trachoma.

The APOC and the OEPA have been successful in coordinating efforts to reduce the transmission of this parasite throughout the world. Currently, there are only limited areas of active transmission remaining.^{6, 7, 11}

As a complementary approach to onchocerciasis control, there have been some efforts to develop recombinant vaccines.^{74, 77} This program, the Onchocerciasis Vaccine for Africa, includes the development of a vaccine containing two antigens, Ov-103 and Ov-RAL-2.^{78–81} Work to create an *Onchocerca* vaccine continues with the hope that even if this vaccine is not completely protective, it will substantially impact endemic areas by reducing host microfilarial loads in children and adolescents.^{80, 82} This would be particularly relevant if ivermectin resistance reaches a threshold impacting transmission of disease.⁸³

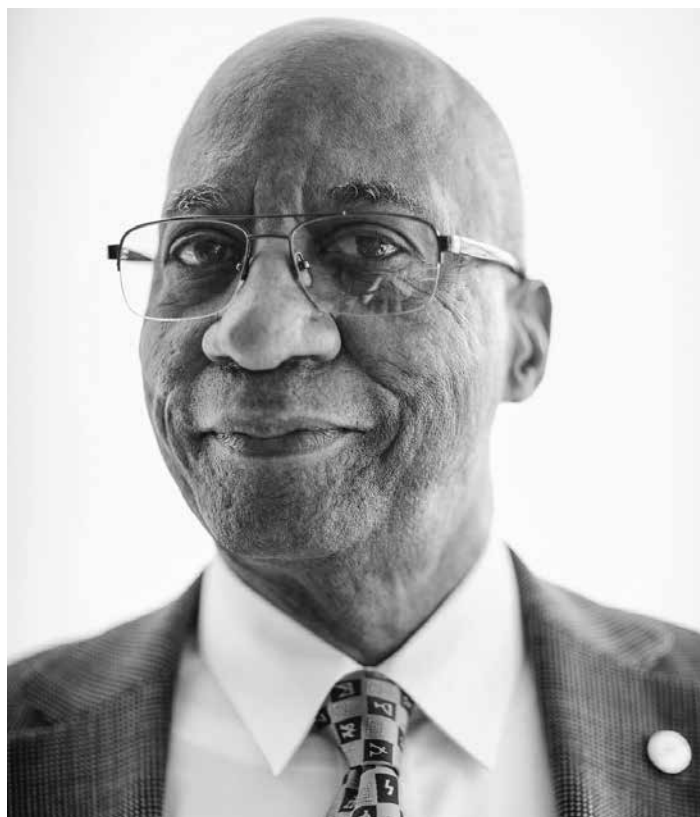
References

1. Otranto, D., et al., Human ocular filariasis: further evidence on the zoonotic role of *Onchocerca lupi*. *Parasit Vectors* **2012**, *5*, 84.
2. Sauerbrey, M., et al., Progress toward elimination of onchocerciasis in the Americas. *Int Health* **2018**, *10* (suppl_1), i71-i78.
3. Greene, B. M., Modern medicine versus an ancient scourge: progress toward control of onchocerciasis. *The Journal of infectious diseases* **1992**, *166* (1), 15-21.
4. Kapoor, U., et al., Onchocercoma in a United Nations Peacekeeper. *Med J Armed Forces India* **2015**, *71* (Suppl 1), S104-6.
5. Abanobi, O. C., et al., Validity of leopard skin manifestation in community diagnosis of human onchocerciasis infection. *Appl Parasitol* **1994**, *35* (1), 8-11.
6. Coffeng, L. E., et al., African Programme For Onchocerciasis Control 1995-2015: model-estimated health impact and cost. *PLoS Negl Trop Dis* **2013**, *7* (1), e2032.
7. Levine, R., et al., The What Works Working Group. *Millions in Global Onchocerciasis in SubSaharan Africa Center for Global Development pp* **2004**, *6* 57-64.
8. Boatin, B., The Onchocerciasis Control Programme in West Africa (OCP). *Ann Trop Med Parasitol* **2008**, *102* Suppl 1, 13-7.
9. Mectizan Donation Program: A Program of The Task Force for Global Health <https://mectizan.org>.
10. Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN) <https://espen.afro.who.int>.
11. Centers for Disease, C.; Prevention, Progress toward elimination of onchocerciasis in the Americas - 1993-2012. *MMWR Morb Mortal Wkly Rep* **2013**, *62* (20), 405-8.
12. Lakwo T, O. D., Ukety T, Post R, Bakajika D., Onchocerciasis Elimination: Progress and Challenges. *Res Rep Trop Med* **2020**, *11*, 81-95.
13. Center, T. C. River Blindness Elimination Program. (accessed June 2).
14. Frallonardo, L., et al., Onchocerciasis: Current knowledge and future goals. *Frontiers in Tropical Diseases* **2022**, *3*.
15. Organization, W. H., Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva. *Licence: CC BY-NC-SA 3.0 IGO* **2020**.
16. WHO, Guidelines for Stopping Mass Drug Administration and Verifying Elimination of Human Onchocerciasis: Criteria and Procedures. Geneva, 2016.
17. O'Neill, J., On the presence of a filaria in "crawcraw." *Lancet* **1875**, *1*, 265-266.
18. Manson, P.; Davidson, A. H., *Filaria volvuloxus*. 1893; Vol. 1016.
19. Robles, R., Enfermedad nueva en Guatemala. *Juventud Med* **1917**, *17* 97-115.
20. Blacklock, D. B., The insect transmission of *Onchocerca volvulus* (Leuckart, 1893): The cause of worm nodules in man in Africa. *British medical journal* **1927**, *1* (3446), 129-33.
21. Schulz-Key, H.; Karam, M., Periodic reproduction of *Onchocerca volvulus*. *Parasitol Today* **1986**, *2* (10), 284-6.
22. Duke, B. O., The population dynamics of *Onchocerca volvulus* in the human host. *Tropical medicine and parasitology : official organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ)* **1993**, *44* (2), 61-8.
23. Burnham, G., Onchocerciasis. *Lancet* **1998**, *351* (9112), 1341-6.
24. Lackey, A., et al., Extracellular proteases of *Onchocerca volvulus*. *Exp Parasitol* **1993**, *68*, 176-185.
25. Capo, V., et al., *Trichinella spiralis*: vascular endothelial growth factor is up-regulated within the Nurse cell during early phase of its formation. **1998**, *84*, 209-214.
26. Hoerauf, A., et al., Onchocerciasis. *BMJ (Clinical research ed.)* **2003**, *326* (7382), 207-10.
27. Keiser, P. B., et al., Bacterial endosymbionts of *Onchocerca volvulus* in the pathogenesis of posttreatment reactions. *The Journal of infectious diseases* **2002**, *185* (6), 805-11.
28. Tawe, W., et al., Angiogenic activity of *Onchocerca volvulus* recombinant proteins similar to vespid venom antigen 5. *Molecular and biochemical parasitology* **2000**, *109* (2), 91-9.

29. Higazi, T. B., et al., Angiogenic activity of an *Onchocerca volvulus* *Ancylostoma*-secreted protein homologue. *Molecular and biochemical parasitology* **2003**, 129 (1), 61-8.
30. Jolodar, A., et al., Molecular cloning of an alpha-enolase from the human filarial parasite *Onchocerca volvulus* that binds human plasminogen. *Biochimica et biophysica acta* **2003**, 1627 (2-3), 111-20.
31. Rougemont, A., et al., Tuberculin skin tests and B.C.G. vaccination in hyperendemic area of onchocerciasis. *Lancet (London, England)* **1977**, 1 (8006), 309.
32. Cooper, P. J., et al., Human onchocerciasis and tetanus vaccination: impact on the postvaccination antitetanus antibody response. *Infection and immunity* **1999**, 67 (11), 5951-7.
33. Prost, A., et al., Lepromatous leprosy and onchocerciasis. *British medical journal* **1979**, 1 (6163), 589-90.
34. Liebau, E., et al., The secretory omega-class glutathione transferase OvGST3 from the human pathogenic parasite *Onchocerca volvulus*. *FEBS J* **2008**, 275 (13), 3438-53.
35. Brattig, N. W., Pathogenesis and host responses in human onchocerciasis: impact of *Onchocerca filariae* and *Wolbachia endobacteria*. *Microbes Infect* **2004**, 6 (1), 113-28.
36. Hise, A. G., et al., Innate immune responses to endosymbiotic *Wolbachia* bacteria in *Brugia malayi* and *Onchocerca volvulus* are dependent on TLR2, TLR6, MyD88, and Mal, but not TLR4, TRIF, or TRAM. *J Immunol* **2007**, 178 (2), 1068-76.
37. Ali, M. M., et al., Immune responses directed against microfilariae correlate with severity of clinical onchodermatitis and treatment history. *The Journal of infectious diseases* **2003**, 187 (4), 714-7.
38. Cooper, P. J., et al., Association of transient dermal mastocytosis and elevated plasma tryptase levels with development of adverse reactions after treatment of onchocerciasis with ivermectin. *The Journal of infectious diseases* **2002**, 186 (9), 1307-13.
39. Pearlman, E., et al., Interleukin 4 and T helper type 2 cells are required for development of experimental onchocercal keratitis (river blindness). *The Journal of experimental medicine* **1995**, 182 (4), 931-40.
40. Gentil, K.; Pearlman, E., Gamma interferon and interleukin-1 receptor 1 regulate neutrophil recruitment to the corneal stroma in a murine model of *Onchocerca volvulus* keratitis. *Infect Immun* **2009**, 77 (4), 1606-12.
41. Timmann, C., et al., Cutaneous pathology in onchocerciasis associated with pronounced systemic T-helper 2-type responses to *Onchocerca volvulus*. *Br J Dermatol* **2003**, 149 (4), 782-7.
42. Timmann, C., et al., Human genetic resistance to *Onchocerca volvulus*: evidence for linkage to chromosome 2p from an autosome-wide scan. *J Infect Dis* **2008**, 198 (3), 427-33.
43. Hoerauf, A., et al., The variant Arg110Gln of human IL-13 is associated with an immunologically hyper-reactive form of onchocerciasis (sowda). *Microbes Infect* **2002**, 4 (1), 37-42.
44. Bari, A. U., Clinical spectrum of onchodermatitis. *J Coll Physicians Surg Pak* **2007**, 17 (8), 453-6.
45. Murdoch, M. E., Onchodermatitis. *Curr Opin Infect Dis* **2010**, 23 (2), 124-31.
46. Murdoch, M. E., et al., A clinical classification and grading system of the cutaneous changes in onchocerciasis. *Br J Dermatol* **1993**, 129 (3), 260-9.
47. Lazarov, A., et al., Pruritus and chronic papular dermatitis in an Ethiopian man. Onchocerciasis (chronic papular onchodermatitis). *Arch Dermatol* **1997**, 133 (3), 382-3, 385-6.
48. Siddiqui, M. A.; al-Khawajah, M. M., The black disease of Arabia, Sowda-onchocerciasis. New findings. *Int J Dermatol* **1991**, 30 (2), 130-3.
49. Richard-Lenoble, D., et al., [Human onchocerciasis and "sowda" in the Republic of Yemen]. *Bull Acad Natl Med* **2001**, 185 (8), 1447-59; discussion 1459-61.
50. Somorin, A. O., Sowda onchocerciasis in Nigeria. *Cent Afr J Med* **1982**, 28 (10), 253-6.
51. Schwartz, D. A., et al., Sowda-type onchocerciasis in Guatemala. *Acta Trop* **1983**, 40 (4), 383-9.
52. Nelson, G. S., Hanging groin and hernia complications of onchocerciasis. *Trans R Soc Trop Med Hyg* **1958**, 52 (3), 272-5.
53. Niamba, P., et al., Hanging groin and persistent pruritus in a patient from Burkina Faso. *Int J Dermatol* **2007**, 46 (5), 485-6.

54. Fuglsang, H., "Leopard skin" and onchocerciasis. *Trans R Soc Trop Med Hyg* **1983**, 77 (6), 881.
55. Edungbola, L. D., et al., 'Leopard skin' as a rapid diagnostic index for estimating the endemicity of African onchocerciasis. *Int J Epidemiol* **1987**, 16 (4), 590-4.
56. Goldman, L.; Figueroa Ortiz, L., Types of dermatitis in American onchocerciasis. *Arch Derm Syphilol* **1946**, 53, 79-93.
57. Babalola, O. E., Ocular onchocerciasis: current management and future prospects. *Clin Ophthalmol* **2011**, 5, 1479-91.
58. Thylefors, B., Ocular onchocerciasis. *Bull World Health Organ* **1978**, 56 (1), 63-73.
59. Colebunders, R., et al., Nodding syndrome and epilepsy in onchocerciasis endemic regions: comparing preliminary observations from South Sudan and the Democratic Republic of the Congo with data from Uganda. *BMC Res Notes* **2016**, 9 (1), 182.
60. Idro, R., et al., Is nodding syndrome an *Onchocerca volvulus*-induced neuroinflammatory disorder? Uganda's story of research in understanding the disease. *Int J Infect Dis* **2016**, 45, 112-7.
61. Olum, S., et al., Nodding syndrome: a concise review. *Brain Commun* **2020**, 2 (1), fcaa037.
62. Boatin, B. A., et al., Detection of *Onchocerca volvulus* infection in low prevalence areas: a comparison of three diagnostic methods. *Parasitology* **2002**, 125 (Pt 6), 545-52.
63. Boatin, B. A., et al., Detection of *Onchocerca volvulus* infection in low prevalence areas: a comparison of three diagnostic methods. *Parasitology* **2002**, 125 (Pt 6), 545-52.
64. Bradley, J. E.; Unnasch, T. R., Molecular approaches to the diagnosis of onchocerciasis. *Advances in parasitology* **1996**, 37, 57-106.
65. Udall, D. N., Recent updates on onchocerciasis: diagnosis and treatment. *Clin Infect Dis* **2007**, 44 (1), 53-60.
66. Kilian, H. D., The use of a topical Mazzotti test in the diagnosis of onchocerciasis. *Tropical medicine and parasitology : official organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ)* **1988**, 39 (3), 235-8.
67. Mand, S., et al., Frequent detection of worm movements in onchocercal nodules by ultrasonography. *Filaria J* **2005**, 4 (1), 1.
68. Greene, B. M., et al., Comparison of ivermectin and diethylcarbamazine in the treatment of onchocerciasis. *The New England journal of medicine* **1985**, 313 (3), 133-8.
69. Burnham, G., Ivermectin treatment of onchocercal skin lesions: observations from a placebo-controlled, double-blind trial in Malawi. *The American journal of tropical medicine and hygiene* **1995**, 52 (3), 270-6.
70. Milton, P., et al., Moxidectin: an oral treatment for human onchocerciasis. *Expert Rev Anti Infect Ther* **2020**, 18 (11), 1067-1081.
71. Chippaux, J. P., et al., Ivermectin treatment of loiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1992**, 86 (3), 289.
72. Hoerauf, A., et al., Doxycycline in the treatment of human onchocerciasis: Kinetics of *Wolbachia* endobacteria reduction and of inhibition of embryogenesis in female *Onchocerca* worms. *Microbes and infection / Institut Pasteur* **2003**, 5 (4), 261-73.
73. Omura, S.; Crump, A., The life and times of ivermectin - a success story. *Nature reviews. Microbiology* **2004**, 2 (12), 984-9.
74. Peters, D. H.; Phillips, T., Mectizan Donation Program: evaluation of a public-private partnership. *Tropical medicine & international health : TM & IH* **2004**, 9 (4), A4-15.
75. Taylor, H. R., et al., Impact of mass treatment of onchocerciasis with ivermectin on the transmission of infection. *Science (New York, N.Y.)* **1990**, 250 (4977), 116-8.
76. Cupp, E. W., et al., The effects of repetitive community-wide ivermectin treatment on transmission of *Onchocerca volvulus* in Guatemala. *The American journal of tropical medicine and hygiene* **1992**, 47 (2), 170-80.
77. Lustigman, S., et al., Towards a recombinant antigen vaccine against *Onchocerca volvulus*. *Trends in parasitology* **2002**, 18 (3), 135-41.
78. Nutman, T. B., Future directions for vaccine-related onchocerciasis research. *Trends in parasitology* **2002**, 18 (6), 237-9.

79. MacDonald, A. J., et al., Ov-ASP-1, the *Onchocerca volvulus* homologue of the activation associated secreted protein family is immunostimulatory and can induce protective anti-larval immunity. *Parasite immunology* **2004**, 26 (1), 53-62.
80. Hotez, P. J., et al., The Onchocerciasis Vaccine for Africa--TOVA--Initiative. *PLoS Negl Trop Dis* **2015**, 9 (1), e0003422.
81. Arumugam, S., et al., Vaccination of Gerbils with Bm-103 and Bm-RAL-2 Concurrently or as a Fusion Protein Confers Consistent and Improved Protection against *Brugia malayi* Infection. *PLoS Negl Trop Dis* **2016**, 10 (4), e0004586.
82. Makepeace, B. L., et al., The case for vaccine development in the strategy to eradicate river blindness (onchocerciasis) from Africa. *Expert Rev Vaccines* **2015**, 14 (9), 1163-5.
83. Zhan, B., et al., Advancing a Human Onchocerciasis Vaccine From Antigen Discovery to Efficacy Studies Against Natural Infection of Cattle With *Onchocerca ochengi*. *Front Cell Infect Microbiol* **2022**, 12, 869039.



Donald Hopkins, M.D., M.P.H. (born 1941)

Hopkins, a Bahamian-American physician, was the director of the health programs at the Carter Center and played a key role in the Guinea Worm eradication efforts and in programs focusing on river blindness, among other neglected tropical parasitic diseases. In addition to devoting his life to reducing the amount of suffering due to parasitic diseases, he is also an acclaimed author; nominated for the Pulitzer prize in 1983. Hopkins is the recipient of several awards and is a leader in the field of applied epidemiology and for his work, he received the Pumphandle Award.

24. *Loa loa*

(Cobbold 1864)

Pronunciation: \lo'ə\\lo'ə\

Introduction

Loa loa (\low-ah\\low-ah\) is a filarial nematode infection found in Central and West Africa, infecting millions of individuals.^{1,2} In some hyperendemic regions, prevalence may be as high as 40%.^{1,3} Loiasis is an emerging infection in areas where the establishment of rubber plantations has altered the rainforest ecology.⁴ *L. loa* infection is seen in returning travelers who spend long periods of time in rural Africa. This includes a diverse array of people with unique occupational exposures, such as anthropologists and individuals involved in ecotourism.⁵ The overwhelming concern for loiasis patients is the severe and adverse reaction in a small percentage of individuals who are recipients of mass drug administration of ivermectin for onchocerciasis. The adult worm lives in subcutaneous tissues. Its main vectors are dipteran flies of the genus *Chrysops* (\KRIS-ops\, \kris-äps\), known as the deer fly.



Figure 24.1. Microfilaria of *Loa loa*. 240 μ m.

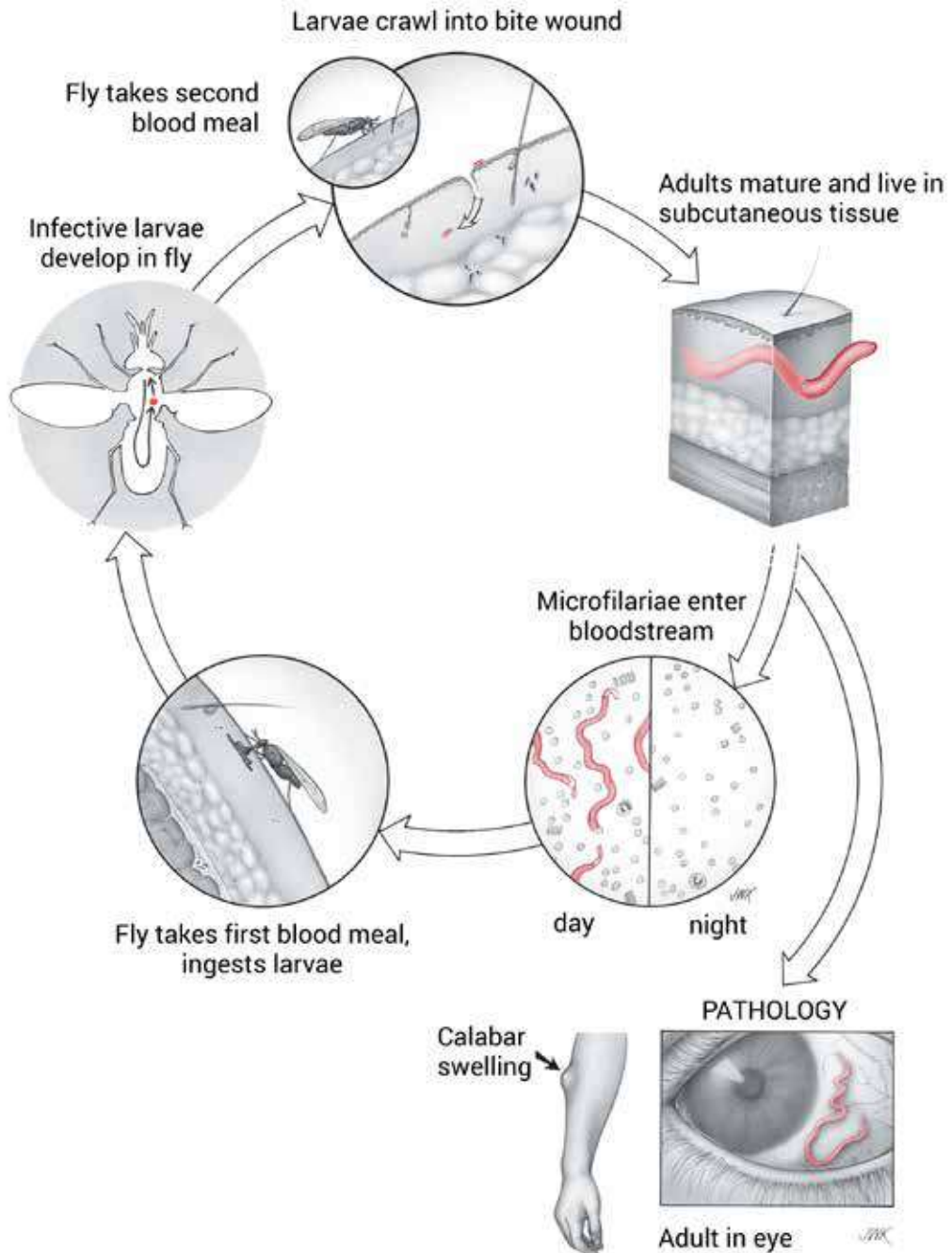
Historical Information

In 1770, M. Mongin, a French surgeon, described a worm passing through the eye of a woman in Santo Domingo, in the Caribbean, and his unsuccessful attempt to remove it.⁶ In 1778, Francois Guyot, a French ship's surgeon, successfully removed a worm from one of the slaves in transit from West Africa to the Americas.⁷ In 1890, Stephen McKenzie, an ophthalmologist, found microfilariae and sent them to Patrick Manson suggesting that they might be the larvae of *Loa loa*.⁸ In 1895, Douglas Argyll-Robertson published the first complete description of the worm and the clinical presentation of the infection.⁹ The woman from whom he removed two adult worms (one of each sex) had lived in Old Calabar (a port city in the area of Africa that is now Nigeria). Swellings in her arms accompanied her infections, and he was the first to describe these swellings in detail.⁷ In 1910, Patrick Manson, along with his colleague George Low, suggested that the swellings were directly connected with infections due to *Loa loa*.^{10,11} These inflammatory lesions are still referred to as Calabar swellings. In 1913, Robert Leiper, described two species of dipterans, *Chrysops dimidiata* and *C. sila-cae*, as the vectors of *L. loa*.¹²

Life Cycle

Female adults measure 60 mm by 0.5 mm; the males are 32 mm by 0.4 mm.¹³ Adult gravid female worms deposit microfilariae (Fig. 24.1), while they wander throughout the subcutaneous tissues. There is 6–12 month prepatent period between the initial bite by an infected fly and the appearance of microfilariae in the blood. Microfilariae (80 μ m by 7 μ m) penetrate capillaries and enter the bloodstream, where they circulate until they become ingested in a blood meal by a *Chrysops* spp. These flies are commonly called deer flies, horse flies, yellow flies, stouts, mango flies, or mangrove flies.¹³⁻¹⁵

Loa loa



L. loa microfilariae exhibit diurnal periodicity that coincides with the feeding habits of *Chrysops*.¹⁶ Larvae penetrate the stomach of the fly, and locate to the fat body. Eight to ten days later, the infective L3 larvae migrate to the cavity of the biting mouthparts and are released into the bite wound when the fly takes another blood meal. The larvae, now in the subcutaneous tissues of the host, develop slowly into adults within 1–4 years. Mature worms mate, and the females begin depositing microfilariae. The adult worms can live in the tissues for up to 17 years.¹⁷

Cellular and Molecular Pathogenesis

In most cases, neither the adult worms in the subcutaneous tissues or the microfilariae in the bloodstream cause any direct pathologic changes. In individuals born and raised in endemic areas, there is evidence for immune tolerance with high levels of circulating microfilariae, low levels of filarial-specific IgG, and few or no clinical symptoms.¹⁸

In temporary residents, there can be peripheral eosinophilia, high levels of filarial-specific IgG, eosinophil activation and degranulation, and localized subcutaneous edema.^{18, 19} The localized swelling, called Calabar swelling, is usually restricted to visitors to endemic areas.^{20, 21} These patients may have exuberant immune responses with parasite-specific IgG and IgE production and proliferation of antigen-specific T cells.^{22, 23} They develop swellings as a local inflammatory reaction in response to filaria antigens released during the migration of adult worms.

A third group of infected individuals who neither have circulating microfilariae nor Calabar swellings, while still living in hyperendemic areas are suspected to have developed protective sterilizing immunity early on.^{24, 25} Unlike other filarial nematodes, there

is no evidence that *L. loa* contains Wolbachia symbionts, based on extensive investigations of light microscopy, electron microscopy, and PCR.^{26, 27}

Clinical Disease

The clinical manifestations of loiasis include Calabar swellings, eye symptoms, encephalitis, cardiomyopathy, renal disease, arthritis, and lymphadenitis. Calabar swellings are 10–20 cm non-erythematous, angioedematous swellings that last a few days. They occur most typically on the extremities and the face, particularly in the periorbital region. The angioedema is often preceded by pain and itching. Recurrences are common. The occasional passage of an adult worm across the subconjunctival space of the eye is perhaps the most disturbing aspect of *L. loa* to most infected individuals.

Patients with more serious complications, including cardiomyopathy, nephropathy, and pleural effusions, have been reported.²¹ The most serious and life-threatening reactions result after patients are exposed to anti-helminthic medications. Some complications occur only after administration of diethylcarbamazine citrate (DEC). These sequelae may result from immune complex deposition. Encephalitis has also been described in patients with very high levels of microfilariae, including microfilariae in the cerebrospinal fluid (CSF).²⁴ Most of these individuals have received DEC or ivermectin while suffering from elevated levels of microfilariae in the blood, and the syndrome probably occurs as part of host immune responses.²⁸ While less common in patients with low levels of microfilariae in the blood, neurological symptoms have been seen with treatment initiation.²⁹ A rare cardiac condition known as endomyocardial fibrosis may result from eosinophilic infiltration of the myocardium in response to *L. loa*.³⁰

Significant differences in the clinical presentation of loiasis occur among long-term visitors to endemic areas, in contrast to people native to these areas.²² People living in endemic regions are often asymptomatic, despite high levels of circulating microfilariae. In contrast, visitors and expatriates suffer from various allergic responses, such as frequent episodes of angioedema, Calabar swellings, hypereosinophilia, and hyper-gammaglobulinemia. This disorder is sometimes called the “hyper-responsive syndrome” of loiasis, reflecting elevated humoral and cell-mediated immune responses to the parasite.²¹

Diagnosis (see Clinical Appendix)

Patients returning from West or Central Africa with localized angioedema or a worm beneath the conjunctiva should be suspected of having loiasis. The Calabar swellings should be clinically differentiated from other causes of angioedema, including complement C1 complex inhibitor deficiency.³¹ Definitive diagnosis is by identifying the microfilariae microscopically in a thin blood smear stained with either Giemsa or Wright’s solution. Among the distinguishing features of *L. loa* microfilariae (Figs. C.50, C.51) are their diurnal periodicity, a sheath, and three or more terminal nuclei.²⁵

With regards to the diurnal periodicity it is important to draw blood for this test during the middle of the day and employ a concentration technique such as the Knott’s test (using formalin), or nucleopore filtration.³² Since visitors to endemic areas often have low levels of circulating microfilariae; serology can be useful in this population.^{31,33,34} Antibodies against *L. loa* of the IgG₄ immunoglobulin subclass may be a reliable marker of infection.³¹ This includes IgG₄ antibodies against the *L. loa* recombinant antigen L1-SXP-1.³⁵ A microsatellite was developed to detect microfilariae in blood by PCR.³⁴

³⁶ Real-time PCR was developed and then adapted for loop-mediated isothermal amplification (LAMP) as a point of care test.^{37,38}

Since levels of circulating microfilariae are critical in decisions regarding treatment, an automated handheld cell counter has been repurposed as a point-of-care test to assess microfilarial levels.³⁹ Alternatively, new blood and urine *L. loa*-specific biomarker tests have been developed.^{40,41}

Treatment (see Clinical Appendix)

The first step in treatment of *Loa loa* infection is based on levels of circulating microfilariae. If levels are greater than 2,500 mf/ml an attempt should be made to lower these levels before treatment with DEC. This may be accomplished through apheresis or several weeks of treatment with albendazole.^{25,42} ⁴³ Once the levels of microfilariae are below 2,500 mf/ml DEC may be administered for 21 days.

DEC destroys all stages of the infecting parasite. The full dose of the drug is not typically started on the first day of treatment. Instead, it is given in a graded manner, beginning with a test dose on day 1 and then increasing the dose to full dose by day 4. This is done to reduce the likelihood of treatment-associated complications, including encephalopathy, that occur as a consequence of mass destruction of *L. loa* microfilariae.²⁹ These iatrogenic complications of loiasis are rare when the microfilarial concentration in blood is less than 2,500 mf/ml at initiation of therapy.²⁵ Antihistamines or corticosteroids may be required to decrease allergic reactions during treatment. In up to 50% of patients, DEC treatment may need to be repeated several times in order to effect a cure.⁴⁴

Adult worms in the eye can be removed surgically. Since they tend not to cause any

pathology it is not a required part of curative therapy. Alternatives to DEC include albendazole, effective in reducing the number of circulating microfilariae by acting directly on adult worms. Ivermectin is not a preferred agent for the treatment of loiasis and can be associated with significant morbidity if given to patients with high levels of circulating microfilariae. Weekly chemoprophylaxis with DEC given in a dose of 300 mg is effective in preventing loiasis among long-term visitors but is not currently recommended for short-term visitors to endemic areas.⁴⁴

Prevention and Control

In hyperendemic regions of Central Africa, 95% of the population has antibodies to *L. loa* antigen by the age of two years.⁴² In the Chaïlu Mountains in the Democratic Republic of Congo, 19% of the native populations are microfilaremic, and more than 50% of the adults have reported sub-conjunctival migrations of an adult worm. Mass or targeted chemotherapy with DEC may reduce transmission in these areas.^{45, 46} Spraying mango groves with insecticides, particularly DDT, remains an effective method for controlling

populations of the vector, since resistance to this insecticide in *Chrysops* has not yet developed.

Widespread use of mass ivermectin drug administration in Sub-Saharan Africa for purposes of controlling lymphatic filariasis (LF) and onchocerciasis has raised concerns about *L. loa* co-infections. The potential complications of unmonitored DEC treatment of loiasis and the risk of encephalopathy are major reasons why this agent is not used routinely. Even the alternative combination of ivermectin and albendazole poses some risk. *L. Loa* encephalopathy is associated with ivermectin treatment of individuals with *L. loa* microfilaremia > 30,000 mf/ml blood, with most of the cases of ivermectin-induced encephalopathy occurring in Cameroon.⁴⁶ In order to reduce the risks associated with ivermectin in this region, albendazole has been evaluated as a possible first-line measure to gradually lower microfilariae burdens.^{47, 48} The recent development of blood and urine tests to detect *L. loa* antigen may represent a future breakthrough in detecting loiasis patients targeted for mass drug administration to combat LF or onchocerciasis.⁴⁰

References

1. Zoure, H. G., et al., The geographic distribution of *Loa loa* in Africa: results of large-scale implementation of the Rapid Assessment Procedure for Loiasis (RAPLOA). *PLoS Negl Trop Dis* **2011**, 5 (6), e1210.
2. Ramharter, M., et al., The African eye worm: current understanding of the epidemiology, clinical disease, and treatment of loiasis. *Lancet Infect Dis* **2024**, 24 (3), e165-e178.
3. Noireau, F., et al., *Loa loa* and *Mansonella perstans* filariasis in the Chaïllu mountains, Congo: parasitological prevalence. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1989**, 83 (4), 529-34.
4. Rodhain, F., [Hypotheses on the dynamic ecology of *Loa* infections]. *Bulletin de la Societe de pathologie exotique et de ses filiales* **1980**, 73 (2), 182-91.
5. Thompson, C., et al., Chronic symptomatic and microfilaremic loiasis in a returned traveller. *CMAJ* **2015**, 187 (6), 437.
6. Mongin, Sur un ver trouve' sous la conjunctive a` Maribarou, isle Saint-Dominique. *J. Med. Chir. Pharm* **1770**, 32, 338-339.
7. Cox, F. E., History of human parasitology. *Clin Microbiol Rev* **2002**, 15 (4), 595-612.
8. Manson, P., The *Filaria sanguinis hominis major* and *minor*, two new species of haematozoa.

- Lancet* **1891**, *i*, 4-8.
9. Argyll-Robertson, D.; Soc, U. K., Case of *Filaria loa* in which the parasite was removed from under the conjunctiva. *Trans Ophthalmol* **1895**, *15*, 137-167.
 10. Low, G. C., Discussion of Manson, P. On the nature and origin of Calabar swellings. *Trans. R. Soc. Trop. Med. Hyg* **1910**, *3*, 251-253.
 11. Manson, P., On the nature and origin of Calabar swellings. *Trans. R. Soc. Trop. Med. Hyg* **1910**, *3*, 244-251.
 12. Leiper, R. T., Report of the Helminthologist for the Half Year Ending 30 April, . Report of the Advisory Commission on Tropical Diseases Research Fund. **1913**, 1913-1914.
 13. Padgett, J. J.; Jacobsen, K. H., Loiasis: African eye worm. *Trans R Soc Trop Med Hyg* **2008**, *102* (10), 983-9.
 14. Horvath, G., et al., Ventral polarization vision in tabanids: horseflies and deerflies (Diptera: Tabanidae) are attracted to horizontally polarized light. *Naturwissenschaften* **2008**, *95* (11), 1093-100.
 15. Baldacchino, F., et al., Tabanids: neglected subjects of research, but important vectors of disease agents! *Infect Genet Evol* **2014**, *28*, 596-615.
 16. Duke, B. O. L.; J., Studies of the biting habits of *Chrysops*. *Trop Med Parasitol* **1955**, *49*.
 17. Eveland, L. K., et al., *Loa loa* infection without microfilaraemia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1975**, *69* (3), 354-5.
 18. Herrick, J. A., et al., Eosinophil-associated processes underlie differences in clinical presentation of loiasis between temporary residents and those indigenous to *Loa*-endemic areas. *Clin Infect Dis* **2015**, *60* (1), 55-63.
 19. Olness, K., et al., Loiasis in an expatriate American child: diagnostic and treatment difficulties. *Pediatrics* **1987**, *80* (6), 943-6.
 20. Nutman, T. B., et al., *Loa loa* infection in temporary residents of endemic regions: recognition of a hyperresponsive syndrome with characteristic clinical manifestations. *The Journal of infectious diseases* **1986**, *154* (1), 10-8.
 21. Nutman, T. B., et al., Immunologic correlates of the hyperresponsive syndrome of loiasis. *The Journal of infectious diseases* **1988**, *157* (3), 544-50.
 22. Kilon, A. D., et al., Loiasis in endemic and non-endemic populations: immunologically mediated differences in clinical presentation. *Dis* **1991**, *163*, 1318-1325.
 23. Limaye, A. P., et al., Regulation of parasite-induced eosinophilia: selectively increased interleukin 5 production in helminth-infected patients. *The Journal of experimental medicine* **1990**, *172* (1), 399-402.
 24. Kilon, A. D.; Einstein, E. M., Pulmonary involvement in loiasis. *Am Rev Respir Dis* **1992**, *145*, 961-963.
 25. Kilon, A. D., et al., Loiasis and *Mansonella* infections In: Tropical Infectious Diseases, Principles, Pathogens, and Practice. Vol 2 eds Churchill Livingstone Pubs New York pp **1999**, 861-872.
 26. Grobusch, M. P., et al., No evidence of *Wolbachia* endosymbiosis with *Loa loa* and *Mansonella perstans*. *Parasitology research* **2003**, *90* (5), 405-8.
 27. Brouqui, P., et al., Doxycycline and eradication of microfilaremia in patients with loiasis. *Emerg Infect Dis* **2001**, *7* (3 Suppl), 604-5.
 28. Gardon, J., et al., Serious reactions after mass treatment of onchocerciasis with ivermectin in an area endemic for *Loa loa* infection. *Lancet* **1997**, *350* (9070), 18-22.
 29. Carme, B., et al., Five cases of encephalitis during treatment of loiasis with diethylcarbamazine. *The American journal of tropical medicine and hygiene* **1991**, *44* (6), 684-90.
 30. Andy, J. J., et al., Loiasis as a possible trigger of African endomyocardial fibrosis: a case report from Nigeria. *Acta tropica* **1981**, *38* (2), 179-86.
 31. Akue, J. P., et al., High levels of parasite-specific IgG4 in the absence of microfilaremia in *Loa loa* infection. *Tropical medicine and parasitology : official organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ)* **1994**, *45* (3), 246-8.
 32. Mak, J. W., Recent advances in the laboratory diagnosis of filariasis. *Malays J Pathol* **1989**, *11*, 1-5.

33. Toure, F. S., et al., IgG4 serology of loiasis in three villages in an endemic area of south-eastern Gabon. *Trop Med Int Health* **1998**, 3 (4), 313-7.
34. Toure, F. S., et al., [Comparative analysis of 2 diagnostic methods of human loiasis: IgG4 serology and nested PCR]. *Bull Soc Pathol Exot* **1999**, 92 (3), 167-70.
35. Klion, A. D., et al., Serum immunoglobulin G4 antibodies to the recombinant antigen, LI-SXP-1, are highly specific for *Loa loa* infection. *The Journal of infectious diseases* **2003**, 187 (1), 128-33.
36. Nutman, T. B., et al., A universally applicable diagnostic approach to filarial and other infections. *Parasitology today (Personal ed.)* **1994**, 10 (6), 239-43.
37. Drame, P. M., et al., Loop-mediated isothermal amplification for rapid and semiquantitative detection of *Loa loa* infection. *J Clin Microbiol* **2014**, 52 (6), 2071-7.
38. Fernandez-Soto, P., et al., Development of a highly sensitive loop-mediated isothermal amplification (LAMP) method for the detection of *Loa loa*. *PloS one* **2014**, 9 (4), e94664.
39. Bennuru, S., et al., Repurposed automated handheld counter as a point-of-care tool to identify individuals 'at risk' of serious post-ivermectin encephalopathy. *PLoS Negl Trop Dis* **2014**, 8 (9), e3180.
40. Geary, T. G., A Step Toward Eradication of Human Filariases in Areas Where *Loa* Is Endemic. *MBio* **2016**, 7 (2).
41. Drame, P. M., et al., Identification and Validation of *Loa loa* Microfilaria-Specific Biomarkers: a Rational Design Approach Using Proteomics and Novel Immunoassays. *MBio* **2016**, 7 (1), e02132-15.
42. Ottesen, E. A., Filarial infections. *Infectious disease clinics of North America* **1993**, 7 (3), 619-33.
43. Kilon, A. D., et al., Albendazole in human loiasis: Results of a double-blind, placebo-controlled trial. *Dis* **1993**, 168.
44. Kilon, A. D., et al., Effectiveness of diethylcarbamazine in treating loiasis acquired by expatriate visitors to endemic regions: long-term follow-up. *Dis* **1994**, 169.
45. Nutman, T. B., et al., Diethylcarbamazine prophylaxis for human loiasis. Results of a double-blind study. *The New England journal of medicine* **1988**, 319 (12), 752-6.
46. Molyneux, D. H., et al., Mass drug treatment for lymphatic filariasis and onchocerciasis. *Trends in parasitology* **2003**, 19 (11), 516-22.
47. Tabi, T.-E., et al., Human loiasis in a Cameroonian village: a double-blind, placebo-controlled, crossover clinical trial of a three-day albendazole regimen. *The American journal of tropical medicine and hygiene* **2004**, 71 (2), 211-5.
48. Tsague-Dongmo, L., et al., Effects of a 3-day regimen of albendazole (800 mg daily) on *Loa loa* microfilaremia. *Annals of tropical medicine and parasitology* **2002**, 96 (7), 707-15.



Susan Lim, Ph.D. (1952–2014)

Susan Lim was a full professor at the University of Malaya and a recognized expert on the Monogenea, a class of ectoparasitic flatworms of fish. As one of the most productive investigators in this field, several parasites were named after her in her honor. Dr. Lim discovered a novel attachment mechanism for monogeneans that involved net-like structures formed by secretions from the attachment organ of opisthaptor.

25. *Dracunculus medinensis*

(Linnaeus 1758)

Pronunciation: \drə-ˈkəŋ-kyə-ləs\

Introduction

Dracunculus medinensis (\dra-KUNG-kyoo-lus\MEH-dee-NEN-sis\), literally “little dragon of Medina” or “the fiery serpent” afflicting the Israelites”, now commonly known as the ‘Guinea worm’ used to occur throughout Central Africa, Yemen, India, Pakistan, and, to a lesser extent, Latin America. In 1986, the World Health Assembly adopted a resolution calling for the eradication of dracunculiasis as part of its initiative to control water-borne infections.¹ The term ‘elimination’ refers to the reduction of disease incidence to zero or close to zero in defined geographic areas, with a requirement for ongoing control efforts, while ‘eradication’ is the complete and permanent worldwide reduction to zero new cases of the disease.² At that time, there were an estimated 3.5 million cases in 20 countries. The prevalence of Guinea worm infection has fallen through a coordinated global eradication campaign spearheaded by a coalition that included the World Health Organization, the Centers for Disease Control and Prevention (CDC), the Carter Center in Atlanta, Georgia, and other agencies. Due to these efforts, in 2014 the

total number of reported cases had decreased from over 3 million to just 126.^{3,5} In 2022, according to the Carter Center, only 13 cases were reported worldwide, with transmission restricted to Chad, Ethiopia, and Mali.

A number of the remaining cases had been in the southern region of the Sudan, where civil conflict and war had limited the access of public health interventions. Remarkably, former President Jimmy Carter negotiated a several-month-long cease-fire in the Sudan to allow eradication efforts to continue.^{6,7} There is hope that the last case of Guinea worm infection will be eradicated by the coming decade, some thirty years after the world was declared free from smallpox.

Infection with *D. medinensis* disfigures the skin and subcutaneous tissues with unsightly scars and can result in serious secondary bacterial infections. Like other neglected diseases, Guinea worm occurs mostly among the poor and disenfranchised in developing countries. Humans are the primary host, and in most areas, the only reservoir for *D. medinensis*. There has been significant confusion regarding other animal reservoirs due to the presence of different dracunculids in cats, dogs, monkeys, horses, cattle, raccoons, foxes and other animals.⁸ There is compelling genetic evidence that *D. medinensis* infects dogs.⁹ The presence of Guinea worm in dogs in Chad is most likely the reason transmission still occurs in that country.^{8,10,11}



Figure 25.1. *Dracunculus medinensis*. The large circular blister, from which the worm is emerging, will heal leaving a disfiguring scar.

Historical Information

The first descriptions of *D. medinensis* infection can be found on papyrus dating back to 1500 BCE.¹² *D. medinensis* worms have been found in Egyptian mummies.¹³ One of the most recognizable features of *D. medinensis* is the treatment for this infection, which involves the removal of the adult female by slowly wrapping it around a stick to extract

Dracunculus medinensis

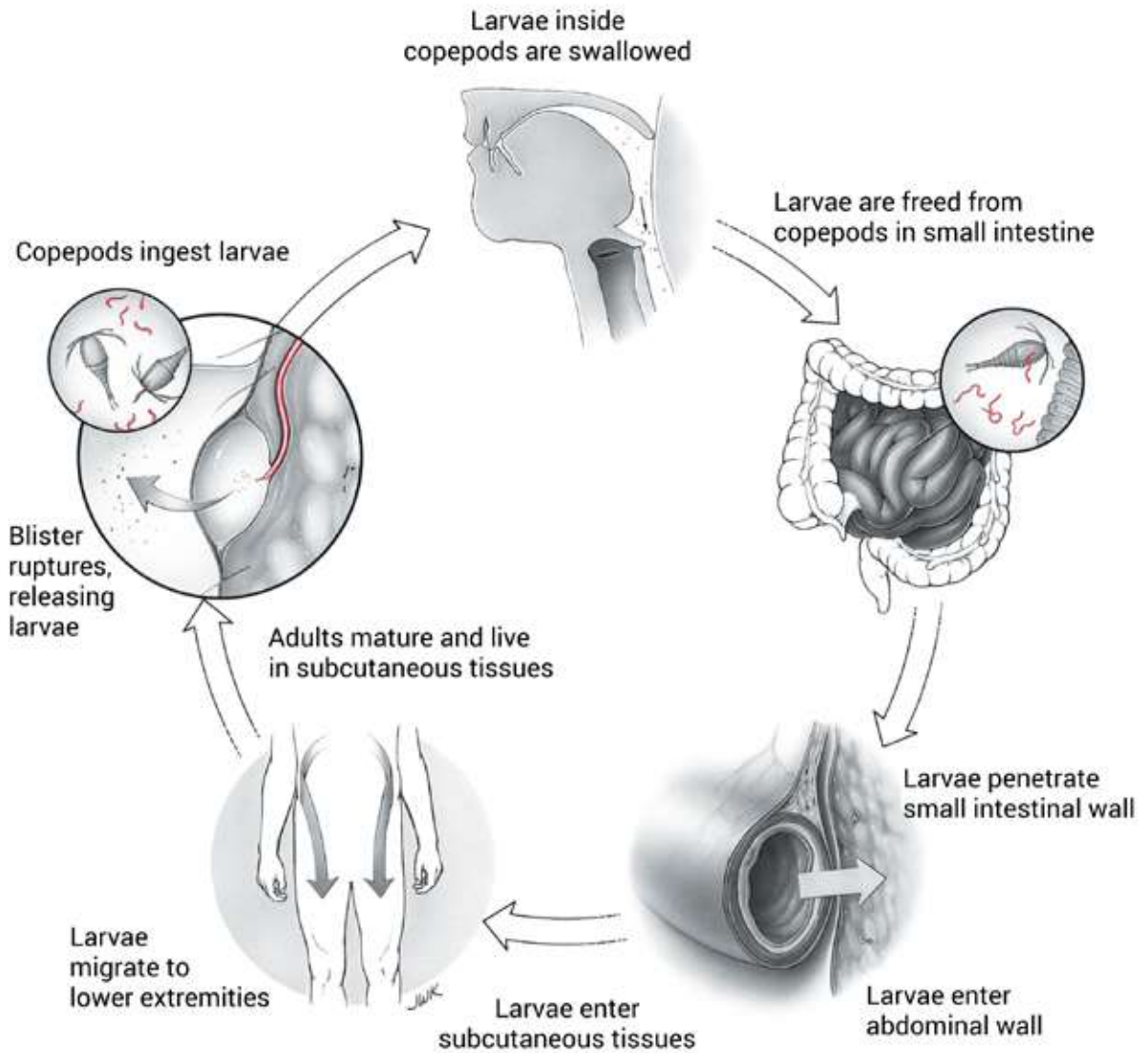




Figure 25.2. Removing an adult *Dracunculus medinensis*. By CDC - This media comes from the Centers for Disease Control and Prevention's Public Health Image Library (PHIL), with identification number #1342.

the worm (Fig. 25.2). The Guinea worm is thought by many to be the serpent depicted on the Rod of Asclepius which has come down through the ages as a symbol of individuals involved in the healing arts.¹⁴ Dracunculiasis is described in the Bible as the “fiery serpent” that afflicted the Israelites.¹⁵

In 1819, Karl Asmund Rudolphi described adult female *D. medinensis* worms containing larvae. In 1838, D. Forbes, a British officer serving in India, described *D. medinensis* in water. In 1849, George Busk published on Guinea worm and suggested that humans become infected through the skin when they are exposed to water containing the contagion.¹⁶ In 1863, Henry Bastian provided the first formal description of the anatomy of *D. medinensis*.¹⁷ In 1870, seven years later, Aleksey Fedchenko reported a partial outline of its life cycle, in which he recognized a crustacean, *Cyclops*, as the intermediate host.¹⁸ *Cyclops*, commonly called water fleas, is the most common genus of freshwater copepods. In 1913, the Indian bacteriologist Dyneshvar Turkhud pieced together all the crucial steps in the life cycle and fulfilled the requirements of Koch's postulates for this infection when he infected human volunteers by having them ingest infected *Cyclops*. He established that infection occurs through ingestion of water contaminated with infected copepods, and

not through direct contact between the skin and contaminated water.¹⁹

Life Cycle

The female adult parasite is long and thin, measuring more than 100 cm by 1.5 mm. The smaller male typically measures 40 mm by 0.4 mm. Some members of the species are red in color, but the reason for this is unknown.²⁰ Adult worms live in the subcutaneous tissues, usually in the lower extremities. Members of both sexes have acutely curved tails that serve to anchor them in the tissues. Humans swallow copepods infected with L3 larvae and become infected. The source is usually untreated drinking water.

The infected copepods are digested in the small intestine, releasing the immature worms. The L3 larvae penetrate the wall of the small intestine and migrate within the connective tissues for up to a year, during which time they molt twice and mature into adults. Approximately one year after ingesting an infected copepod, the fertilized female Guinea worm finishes her migration to the subcutaneous tissues. She induces the formation of a papule that evolves into a fluid-filled vesicle or blister. This vesicle forms so that it surrounds the worm's anterior vulva. Vesicles usually develop in the lower extremity, particularly the foot, but can also develop in several other locations.²¹ A severe burning sensation develops at its location, prompting the infected individual to submerge their limb or foot into water (e.g., step wells, slow-moving rivers or lakes) to find relief from the pain.¹⁴ When the vesicle comes in contact with fresh water, it ruptures, inducing the worm to undergo prolapse of its uterus. Motile L1 larvae are released into the vulvar cavity and then into the water.²² The active larvae are mistaken for zooplankton by the copepods and they ingest them. Copepods of many genera can serve as intermediate hosts,

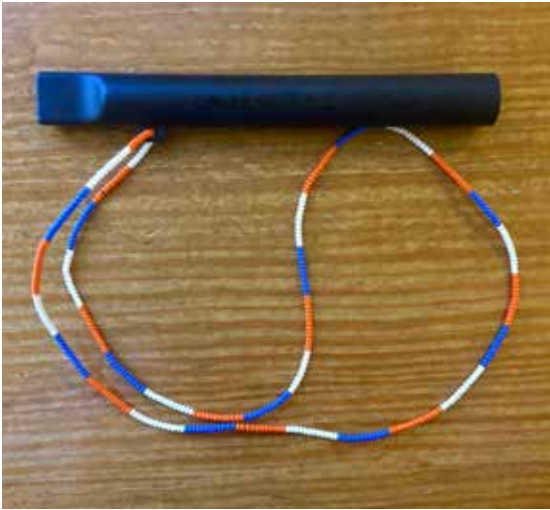


Figure 25.3. Filter straw used to strain infected copepods out of drinking water to prevent infection.

including Cyclops, Mesocyclops, and Thermocyclops.

The larvae rapidly penetrate the hemocoel of the crustacean and develop within 2–3 weeks into infective L3 larvae. The life cycle is completed when a human or dog ingests infected crustaceans.^{10, 11}

Cellular and Molecular Pathogenesis

During primary infection, there are no apparent host responses to the presence of the worm during its maturation process. If worms do not complete their migration and die, they may either disintegrate or become calcified. This causes disease if the calcification event happens to occur near a joint. At the conclusion of a successful migration, the worm secretes a toxin that induces local inflammation, leading to a papule that evolves into a blister and ultimately forms an ulcer. Just before the formation of the blister, infected individuals can develop systemic symptoms, including fever, nausea, vomiting, and diarrhea.²³

Ulcers occur most frequently on the lower part of the body (legs and feet), but can also

locate on the upper extremities and the trunk. Patients can become sensitized to worm secretions, with the consequent allergic reactions of urticaria and pruritus. Anaphylactic reactions have also been reported. If left untreated, ulcers often become secondarily infected, leading to tetanus, gangrene, and even death. Historically, about 1% of the cases worldwide were fatal due largely to bacterial super-infection and death from septicemia.²⁴

Clinical Disease

Multiple cutaneous blisters and ulcers are characteristic manifestations of infection with *D. medinensis* in endemic areas (Fig. 25.1). Allergic reactions usually occur in advance of the rupture of the blister or with attempts to remove the worm. Dracunculiasis is not usually fatal but causes substantial disability (3–10 weeks) in nearly half of the affected individuals. In Nigeria, dracunculiasis was responsible for 25% of the absenteeism in infected school children.²⁵ Secondary bacterial infections are common, with cellulitis spreading along the track of the worm. Ankylosing of joints, arthritis, or contractures can develop and lead to permanent disability.²⁵ In Nigeria, it was estimated that Guinea worm infection in adults resulted in an average of 100 work days lost per year.²⁶

Diagnosis (see Clinical Appendix)

Definitive diagnosis is achieved by locating the head of the adult worm in the skin lesion or by identifying the larvae that are released into freshwater. Radiographs may reveal calcifications corresponding to the adult worms in the subcutaneous tissues. There is a reliable ELISA test for *D. medinensis*, but its availability is limited.²⁷

Treatment (see Clinical Appendix)

The time-honored therapy involves winding the worm on a thin stick until it is extracted (Fig. 25.2). This must be done slowly to ensure the worm does not break. Surgical removal of the worms has been effective but may exaggerate allergic reactions. Mebendazole treatment or the use of other antihelminthic therapy for dracunculiasis is not recommended.²⁸ Wound care and pain management are important components of caring for patients with dracunculiasis.

Prevention and Control

Successful eradication of dracunculiasis has been possible because: 1. there is no human carrier state (beyond a 1-year incubation period), 2. there are few significant animal reservoirs, 3. transmission is seasonal, 4. cases are easily detected by observing individuals with protruding worms or circular scars from previous infections, 5. the methods for controlling transmission are relatively simple.^{29,30} Recently, it has been noted that the other animals, including domestic dogs and

cats in Chad and olive baboons in Ethiopia, may serve as animal reservoirs for Guinea worm. This complication could thwart global eradication efforts.⁹⁻¹¹

The major approaches to Guinea worm prevention and eradication include: 1. filtering drinking water through finely-woven cloth to mechanically remove copepods or drinking water through filter straws (Fig. 25.3), 2. treating contaminated water with a larvicide known as temephos (ABATE®), 3. health education to prevent infected individuals from entering drinking water sources when Guinea worms are emerging, and 4. providing clean water from wells.³ The successes of this approach have resulted in international acclaim and in large measure, reflect extraordinary disease control efforts by the World Health Organization and CDC, together with advocacy efforts personally championed by former President Jimmy Carter, Dr. Donald Hopkins, Dr. Ernesto Ruiz-Tiben and the staff of the Carter Center. Guinea worm eradication may be the most impressive accomplishment a former U.S. President has ever achieved.

References

1. Despommier, D. D., *People, parasites, and plowshares : learning from our body's most terrifying invaders*. Columbia University Press: 2014; p xxi, 213 pages.
2. Hotez, P. J., et al., Combating tropical infectious diseases: report of the Disease Control Priorities in Developing Countries Project. *Clin Inf Dis* **2004**, *38* (6), 871-8.
3. Mmwr, Centers for Disease Control and Prevention (CDC). Progress toward eradication of drancunculiasis, . *Mortal Wkly Rep* : 871-2. **2004**, *53* (37), 2002-2003.
4. Hopkins, D. R., et al., Progress Toward Global Eradication of Dracunculiasis, January 2014-June 2015. *MMWR Morb Mortal Wkly Rep* **2015**, *64* (41), 1161-5.
5. Al-Awadi, A. R., et al., Guinea worm (Dracunculiasis) eradication: update on progress and endgame challenges. *Trans R Soc Trop Med Hyg* **2014**, *108* (5), 249-51.
6. Implementation of health initiatives during a cease fire-Sudan, . *MMWR*. **1995**, *44*, 433-436.
7. Imported dracunculiasis-United States, and . *MMWR* **209**. **1998**, 47.
8. Bimi, L., et al., Differentiating *Dracunculus medinensis* from *D. insignis*, by the sequence analysis of the 18S rRNA gene. *Ann Trop Med Parasitol* **2005**, *99* (5), 511-7.
9. Thiele, E. A., et al., Population genetic analysis of Chadian Guinea worms reveals that human and non-human hosts share common parasite populations. *PLoS Negl Trop Dis* **2018**, *12* (10), e0006747.
10. Callaway, E., Dogs thwart effort to eradicate Guinea worm. *Nature* **2016**, *529* (7584), 10-1.

11. Eberhard, M. L., et al., The peculiar epidemiology of dracunculiasis in Chad. *Am J Trop Med Hyg* **2014**, *90* (1), 61-70.
12. Cox, F. E., History of human parasitology. *Clin Microbiol Rev* **2002**, *15* (4), 595-612.
13. Nunn, J. F.; Tapp, E., Tropical diseases in ancient Egypt. *Trans R Soc Trop Med Hyg* **2000**, *94* (2), 147-53.
14. Awofeso, N., Towards global Guinea worm eradication in 2015: the experience of South Sudan. *Int J Infect Dis* **2013**, *17* (8), e577-82.
15. Kuchenmeister, F., Animal and vegetable parasites. *The Sydenham Society, London, United Kingdom*. **1857**.
16. Busk, G., Observations on the Structure and Nature of the *Filaria medinensis*, or Guinea Worm. *Trans. Microsc. Soc.* **1864**, *2*, 65-80.
17. Bastian, H. C., On the structure and the nature of *Dracunculus*, or Guinea worm. *Trans Linn Soc* **1863**, *24*, 101-134.
18. Fedehenko, A. P., Concerning the structure and reproduction of the guinea worm (*Filaria medinensis*, L). Izv Imper Obscuh Liubit Estes. *Anthrop Ethnog* **1870**, *8*, 71-81.
19. Turkhud, D. A.; Major, W., Report of the Bombay Bacteriological Laboratory for the Year. *Report presented by Glen Liston Government Central Press Bombay pp* **1914**, 14-16.
20. Eberhard, M. L., et al., Red *Dracunculus medinensis*. *Am J Trop Med Hyg* **1989**, *41* (4), 479-81.
21. Karam, M.; Tayeh, A., Dracunculiasis eradication. *Bull Soc Pathol Exot* **2006**, *99* (5), 377-85.
22. Ruiz-Tiben, E., et al., Progress toward the eradication of dracunculiasis (Guinea worm disease). *Emerg Infect Dis* **1995**, *1* (2), 58-60.
23. Greenaway, C., Dracunculiasis (guinea worm disease). *CMAJ* **2004**, *170* (4), 495-500.
24. Cairncross, S., et al., Dracunculiasis (Guinea worm disease) and the eradication initiative. *Clin Microbiol Rev* **2002**, *15* (2), 223-46.
25. Llegbodu, V. A., et al., Impact of guinea worm disease on children in Nigeria. *Am Med Hyg* **1986**, *35*, 962-964.
26. Kale, O. O., The clinico-epidemiological profile of guinea worm in the Ibadan district of Nigeria. *Am J Trop Med Hyg* **1977**, *26* (2), 208-14.
27. Klicks, M. M., et al., Development of a rapid ELISA for early sero-diagnosis of dracunculosis. *Dis* **1984**, *16*, 287-294.
28. Chippaux, J. P., Mebendazole treatment of dracunculiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1991**, *85* (2), 280.
29. Hopkins, D. R.; Ruiz-Tiben, E., Strategies for dracunculiasis eradication. *Bulletin of the World Health Organization* **1991**, *69* (5), 533-40.
30. Hopkins, D. R.; Foege, W. H., Guinea worm disease (letter to the editor). *Science* **1981**, 212-495.

26. Other Nematodes of Medical Importance

Several nematode infections of low to moderate prevalence can present with serious clinical consequences when they occur and deserve mention. Table 26.1 lists their geographic distribution, major pathologic effects, infection modes, diagnosis methods, and therapies. A brief description of those preceded by an asterisk in the table is given in the text below.

Dirofilaria immitis

(Leidy 1856)

and other *Dirofilaria* spp.

Pronunciation: \dī-rō-fə-'lar-ē-ə\

Dirofilaria immitis (\DYE-row-fi-LAR-ee-a\), the dog heartworm, is an accidental parasite of humans, usually infecting the lungs where it produces solitary nodules. The pulmonary lesions probably result from a dead worm being washed into the pulmonary artery from the right ventricle, followed by embolization to the lung.¹ These nodules are frequently diagnosed on chest radiographs as a “coin lesion” that mimics lung carcinoma. The diagnosis of pulmonary dirofilariasis is usually made after finding a calcified worm in a granuloma in the resected lesion. The bite of an infected mosquito is the only way this parasite can be transmitted.²

There is concern that climate change is increasing the incidence of *Dirofilaria* spp. in animals and humans.² Several cases have been identified in the United States, particularly Texas, Florida, and Louisiana. This pathogen has now spread as far north as Alaska.^{1,3} The seroprevalence in humans or other animals can be estimated by immunodiagnostic tests using excretory-secretory products or somatic antigens from the adult

worm.⁴ *Dirofilaria tenuis*, a parasite of raccoons and related *Dirofilaria* spp., causes zoonotic subcutaneous infections in humans that result in discrete nodules.⁵ The diagnosis is typically established when the parasite is noted in a histopathologic section of tissue. Some patients have circulating eosinophilia.

Capillaria hepatica

(Bancroft 1893)

Pronunciation: \kap-ə-'lar-ē-ə\ hi-'pa-ti-kə\

The genus *Capillaria* (\cap-il-lar-ia\) has four members capable of infecting humans: *C. philippinensis*, *C. hepatica*, *C. plica*, and *C. aerophila*.⁶ Only *C. philippinensis* is a significant regional public health problem. *C. hepatica* is a parasite of rodents, mainly rats, that can, on rare occasions, infect humans.⁷ Human cases have been reported in Asia, South America, North America, and Europe.⁷ ⁸ Adult worms live and lay eggs in the liver parenchyma within a syncytium. These eggs are released into the environment once the infected animal dies or is eaten by a predator. Once released into the environment, eggs embryonate and become infective for another host. Infective eggs are ingested and hatch in the small intestine. Eventually, larvae mature into adults and reach the liver. After mating, the females deposit eggs, completing the life cycle. The liver serves as the source of nourishment for the adult parasites. The host may suffer liver failure and die if enough worms are present. Rarely this pathogen is transmitted to humans by accidentally ingesting embryonated eggs.

Diagnosis used to require liver biopsy and the visualization of adults or eggs in the samples, but serological testing is now available.⁹ Often, the disease is unsuspected and discovered only at autopsy or incidentally through liver biopsy. Alternatively, it can pres-

ent with abdominal lymphadenopathy and eosinophilia.¹⁰ Occasionally, patients may be asymptomatic and pass *C. hepatica* eggs in their stools, but this is felt to be ingested eggs passing through the intestinal tract, as infection does not result from the release of eggs into the feces. (Fig. C.55) Numerous treatment regimens, including disophenol (2-6-diiodo-4-nitrophenol), albendazole, and prednisone, are effective strategies for managing this infection.¹¹⁻¹³

Capillaria philippinensis

(Chitwood, Velasquez, and Salazar 1968)

Pronunciation: \kap-ə-'lar-ē-ə\\ fil-ləp-in-en-sis\

Infection with *Capillaria philippinensis* occurs mainly in parts of Thailand and the Philippines, where infection can lead to death.¹⁴ Several deaths were associated with an outbreak of chronic gastroenteritis in central Luzon. Cases have also been reported in Japan, Taiwan, and Korea, and the infection has emerged in Egypt.¹⁵

Adult worms resemble *Trichinella spiralis* in size (Fig. 26.1) and general biology. Like *Trichinella* and *Trichuris*, the adults have an attenuated anterior end with an esophagus surrounded by a row of secretory cells called stichocytes.⁶ The worms locate to the intracellular compartment of the columnar epithelium of the small intestine and deposit living larvae there, which are infectious within the same host. In this respect, its biology mimics the auto-infectious cycle of *Strongyloides stercoralis*. In contrast, no evidence exists that patients harboring *C. philippinensis* are immunosuppressed. As infection progresses, the patient first begins passing embryonated, then unembryonated eggs in the stools.

C. philippinensis is most likely a parasite of waterfowl that feed on fish, which are



Figure 26.1. Adults of *Capillaria philippinensis*. The female is 3 mm x 45 μ m, and the male is 2.5 mm x 30 μ m.

the intermediate hosts for this nematode. Humans become infected by eating raw or undercooked infected fish. In the Philippines, ingestion of “jumping salad,” which consists of leafy green vegetables and a variety of live aquatic animals, including shrimp and minnows of various species, is thought to be a common source of this infection.

The clinical disease consists of rampant diarrhea associated with malaise, anorexia, and vomiting. Patients frequently develop a protein-losing enteropathy and malabsorption of fats and carbohydrates, which, in turn, leads to a wasting syndrome. Patients who are ill for several months without treatment develop profound electrolyte imbalance.⁶ Death results from cachexia, heart failure, and secondary bacterial infections. The mortality rate approaches 10% in some endemic areas. At autopsy, numerous *C. philippinen-*

Capillaria philippinensis

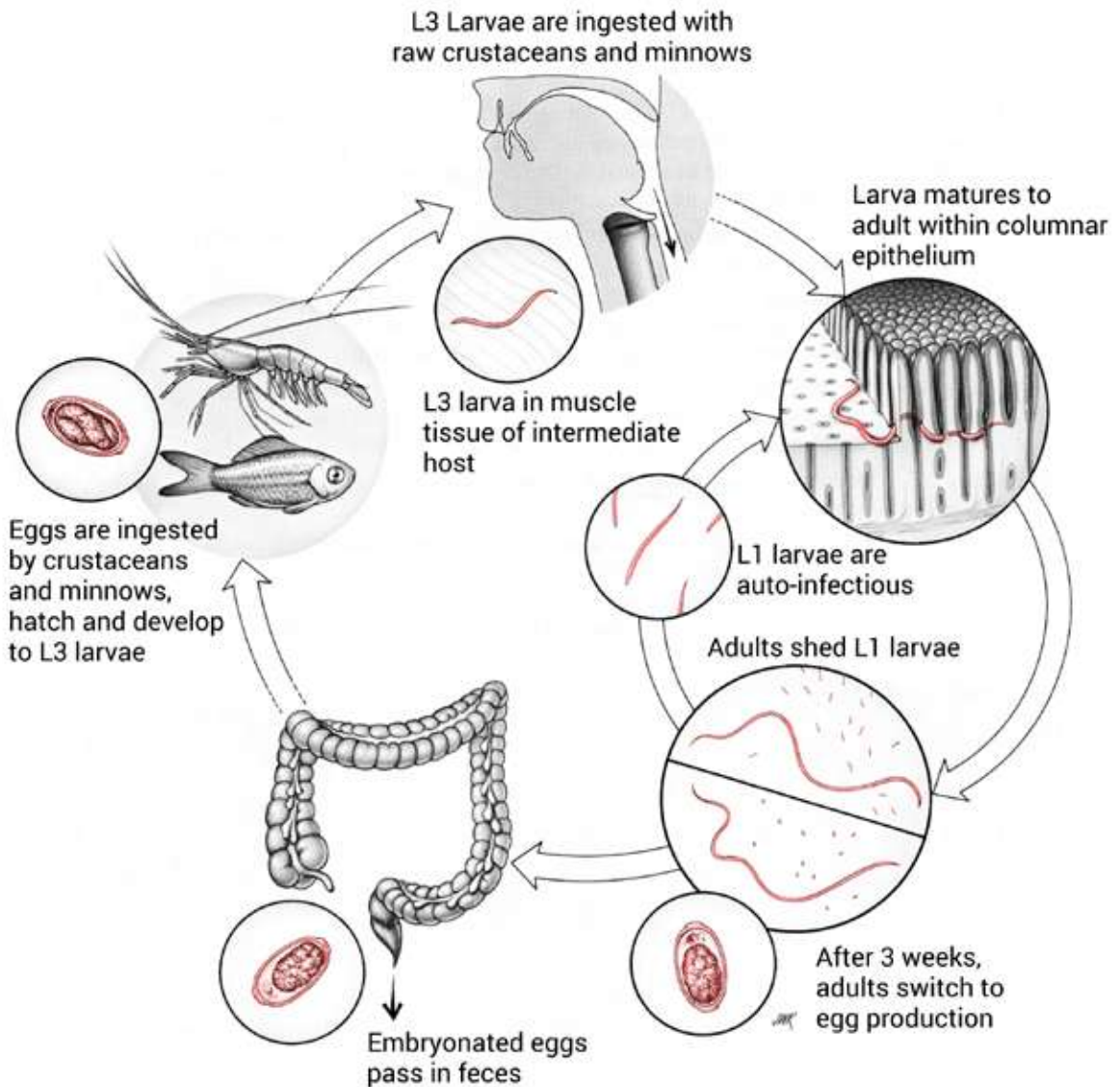


Table 26.1. Other nematodes of medical importance

Parasite	Geographic distribution	Major pathologic consequences	Mode of infection	Diagnosis
* <i>Capillaria hepatica</i>	Worldwide	Necrotic lesions in liver	Oral route	Biopsy
* <i>Capillaria philippinensis</i>	Philippines	Malabsorption syndrome, diarrhea	Oral route	Stool examination for larvae and eggs
<i>Diocotophyma renale</i>	North and South America, China	Complete destruction of infected kidney	Oral route	Urine examination for eggs
* <i>Dirofilaria immitis</i>	Worldwide	Pulmonary nodules	Bite of infected mosquito	Biopsy
* <i>Mansonella ozzardi</i>	Central and South America, Caribbean	Chronic arthritis	Bite of infected midge	Blood smear or Knott's test for microfilariae
* <i>Mansonella perstans</i>	Central and South America, Africa	None	Bite of infected midge	Blood smear or Knott's test for microfilariae
* <i>Mansonella streptocerca</i>	Africa	Pruritic dermatitis	Bite of infected midge	Impression smear of skin snip for microfilariae
* <i>Oesophagostomum bifurcum</i>	West Africa	Intestinal nodules	Oral route	Stool examination for eggs
<i>Syngamus laryngeus</i>	South America, Caribbean, Philippines	Asthma, hemoptysis	Unknown	Sputum or stool examination for eggs
* <i>Ternidens diminutus</i>	Africa	Iron-deficiency anemia	Unknown	Stool examination for eggs
<i>Trichostrongylus</i> spp.	Worldwide	Anemia	Oral route	Stool examination for eggs

* Discussed in the text.

sis worms can be identified at all stages in the lumen and intestinal mucosa. Due to auto-infection, as many as 200,000 worms have been recovered in 1 liter of bowel fluid.⁶

Diagnosis depends upon finding eggs or larvae in feces or detecting the parasite on small intestine biopsy. They are usually present in patients with abdominal pain, diarrhea, and weight loss. The eggs resemble those of *Trichuris trichiura* (Fig. C.58). Mebendazole or albendazole are effective treatments for *C. philippinensis*.⁶ Albendazole is preferable, as the drug appears to act on larvae and adult worms, and relapses have not yet been reported.^{14, 15} Because of the risk of autoinfection, all infected patients should be treated.

During the epidemic in central Luzon, the lagoons were contaminated with bed sheets soiled with feces from infected patients.⁶ This situation helped propagate the life cycle in fish and other intermediate hosts. To prevent infection, it is recommended that raw or undercooked fish be avoided. Cultural eating habits are, however, challenging to change.

Mansonella ozzardi (Manson 1897)

Pronunciation: \man-sə-'nel-ə\äz-zar-dē\

Mansonella ozzardi (MAN-so-nel-ah\ OZ-zar-DEE\)) is a filarial infection found throughout South America, Central America, and the Caribbean islands, especially Haiti. In some highly endemic regions, up to 70% of the population may harbor circulating microfilariae.¹⁷⁻²¹ Vectors include biting midges and black flies of the genus *Simulium*.²² Adult worms are located in the visceral adipose tissue, the peritoneal or thoracic cavities, or lymphatics. Microfilariae are non-periodic and possess a characteristic sharp tail. They can be found circulating in the bloodstream. This infection may produce allergic-type symptoms, such as urticaria and lymphadenopathy, although it usually results in an asymptomatic circulating eosinophilia. This infection has been implicated as a possible cause of chronic arthritis. Diagnosis depends upon finding the microfilariae on a stained blood smear or in a skin sample. Serologi-

cal testing is available but lacks specificity. A PCR test has been developed but is not widely available. Ivermectin is the treatment of choice for *M. ozzardi* infection and has been shown to reduce the number of circulating microfilariae and symptoms significantly.²³⁻²⁶ Neither diethylcarbamazine citrate (DEC) nor benzimidazoles are effective against *M. ozzardi*.^{24, 27}

Mansonella perstans

(Manson 1891)

Pronunciation: \man-sə-'nel-ə\\pər-stans\

Mansonella perstans (MAN-so-nel-ah) is a filarial parasite found in Africa, northeastern South America, and parts of the Caribbean. It is transmitted from person to person by biting midges. In Africa, gorillas and chimpanzees may be significant reservoirs. High prevalence rates have been reported in rural Senegal.²⁸ Adults live freely in serous cavities such as the pleural, pericardial, or peritoneal cavities, producing microfilariae that circulate in blood. *M. perstans* infection is usually asymptomatic, but it is known to cause painless nodules in the conjunctiva with swelling of the eyelids in Africa, where it is known as the Ugandan eye worm or Kampala eye worm.²⁹ The organism may also result in symptoms similar to *Loa loa* infection, such as angioedema and Calabar swellings. Microfilariae of *M. perstans* may be observed in peripheral blood and can be demonstrated by examining a stained blood smear or the Knott test. Serological testing is available but lacks specificity. A PCR test has been developed but has yet to be widely available.

Most standard antihelmintic therapies have been ineffective for *M. perstans*.³⁰⁻³² The discovery that this filarial parasite harbors Wolbachia suggested that doxycycline might be an effective treatment option.³³⁻³⁵ Subsequent

use of doxycycline has proven to be a highly effective therapy in species from Mozambique and the Democratic Republic of Congo that harbor Wolbachia.³⁶ Combination therapy has been successful in some cases.³⁷ Co-infections with *M. perstans* do not appear to significantly alter post-treatment reaction profiles to single-dose ivermectin/albendazole for patients with lymphatic filariasis (LF).³⁸

Mansonella streptocerca

(Macfie and Corson 1922)

Pronunciation: \man-sə-'nel-ə\\strep-tə-'sər-kə\

Mansonella streptocerca (\MAN-so-nel-ah\\STREP-tow-ser-ka\)) is a filarial parasite in tropical rainforests in Central Africa. Adult worms locate to the subcutaneous tissues, as do microfilariae.^{39, 40} Its vectors are biting midges. The primary clinical manifestation is pruritic dermatitis with hypopigmented macules that may resemble onchocerciasis. There is often an associated axillary or inguinal lymphadenopathy. Diagnosis is made by microscopically identifying microfilariae in specimens of skin or impression smears made from them. Serological testing is available but lacks specificity. A PCR test has been developed to aid in detecting microfilariae in skin biopsy specimens.⁴¹ Microfilariae must be differentiated from those of *Onchocerca volvulus*, usually by attempting to identify the characteristic hook-shaped tail, which is sometimes referred to as a “shepherd’s crook.”⁴² DEC is the drug of choice, but it may exacerbate pruritus, in which case, anti-inflammatory agents and antihistamines may be necessary. Although ivermectin may also be effective, especially against microfilariae, but not against adult worms, some concerns about side effects exist.^{43, 44}

Oesophagostomum bifurcum

(Creplin 1849)

Pronunciation: \i-,säf-ə-'gäs-tə-məm\

Oesophagostomum bifurcum (I-sof-ah-GOS-tow-mum) is a nematode mainly infecting non-human primates in Africa and Asia. *O. bifurcum* infects up to 30% of these human populations in northern Togo and north-eastern Ghana, with an estimated 250,000 cases.⁴⁵⁻⁴⁸ Sporadic cases have also been described in other parts of Africa, Asia, and South America.⁴⁷ Adults 30–40 years of age have the highest prevalence in these regions. These nematodes are often called nodular worms because they cause nodule formation on the wall of the intestine.

Adult worms produce about 5,000 eggs per day, which pass with feces and mature into infective L3 larvae in soil.⁴⁹ Eggs morphologically resemble those of hookworms. Humans are infected when they ingest infective larvae, which then penetrate the small intestinal wall and develop into adults. Some patients develop multi-nodular disease, while, in others, a single nodular mass develops.⁴⁶ The nodules in male patients are larger than the ones in females.⁴⁶ The nodular disease of *Oesophagostomum* infection often presents as an abdominal mass, which can be painful and mimics a surgical abdo-

men. Usually, the mass is asymptomatic. As a result, the infection is frequently diagnosed at biopsy, although ultrasound is also of great value.⁵⁰ A fecal examination is the diagnostic method of choice in individual patients (eggs cannot be visually distinguished from hookworm eggs). At the same time, PCR has been developed and is used in prevalence studies.⁵¹ Pyrantel pamoate is the recommended drug for treating infections due to *O. bifurcum*, and albendazole is also effective.⁵² Surgical resection of the nodules is sometimes necessary.

Ternidens diminutus

Pronunciation: \tər-nə-,denz\

Ternidens diminutus (ter-nah-DENZ) is a nematode capable of infecting humans that resembles *O. bifurcum*. *Ternidens* eggs resemble hookworm eggs, so *T. diminutus* is sometimes called “the false hookworm.”⁵³ It is primarily a parasite of non-human primates but has been demonstrated to cause human infections in Zambia, Zimbabwe, Tanzania, and Asia.^{54, 55} *T. diminutus* can result in colonic ulcerations and nodular lesions, but there are usually few symptoms. Both pyrantel pamoate and thiabendazole have been used to treat patients, and other benzimidazoles may also be effective.^{53, 56, 57}

References

1. Asimacopoulos, P. J., et al., Pulmonary dirofilariasis. The largest single-hospital experience. *Chest* **1992**, *102* (3), 851-5.
2. Simon, F., et al., What is new about animal and human dirofilariosis? *Trends Parasitol* **2009**, *25* (9), 404-9.
3. Diaz, J. H.; Risher, W. H., Risk factors for human heartworm infections (dirofilariasis) in the South. *J La State Med Soc* **2015**, *167* (2), 79-86.
4. Akao, N., et al., Immunoblot analysis of *Dirofilaria immitis* recognized by infected humans. *Annals of tropical medicine and parasitology* **1991**, *85* (4), 455-60.
5. Orihel, T. C., et al., Subcutaneous dirofilariasis: single inoculum, multiple worms. *The American journal of tropical medicine and hygiene* **1997**, *56* (4), 452-5.
6. Cross, J. H., Intestinal capillariasis. *Clinical microbiology reviews* **1992**, *5* (2), 120-9.
7. Li, C. D., et al., *Capillaria hepatica* in China. *World J Gastroenterol* **2010**, *16* (6), 698-702.

8. Wang, Z., et al., The emerging but neglected hepatic capillariasis in China. *Asian Pac J Trop Biomed* **2013**, 3 (2), 146-7.
9. Juncker-Voss, M., et al., Serological detection of *Capillaria hepatica* by indirect immunofluorescence assay. *J Clin Microbiol* **2000**, 38 (1), 431-3.
10. Sharma, R., et al., Capillaria hepatica infection: a rare differential for peripheral eosinophilia and an imaging dilemma for abdominal lymphadenopathy. *Ann Parasitol* **2015**, 61 (1), 61-4.
11. McQuown, A. L.; J., *Capillaria hepatica*: reported genuine and spurious cases. *Am Med Hyg* **1950**, 30, 761-767.
12. Nabi, F., et al., *Capillaria hepatica* infestation. *Indian Pediatr* **2007**, 44 (10), 781-2.
13. Pereira, V. G.; Mattosinho Franca, L. C., Successful treatment of *Capillaria hepatica* infection in an acutely ill adult. *Am J Trop Med Hyg* **1983**, 32 (6), 1272-4.
14. el-Karakasy, H., et al., *Capillaria philippinensis*: a cause of fatal diarrhea in one of two infected Egyptian sisters. *Journal of tropical pediatrics* **2004**, 50 (1), 57-60.
15. Bair, M.-J., et al., Clinical features of human intestinal capillariasis in Taiwan. *World journal of gastroenterology : WJG* **2004**, 10 (16), 2391-3.
16. Limsrivilai, J., et al., Intestinal capillariasis in the 21st century: clinical presentations and role of endoscopy and imaging. *BMC Gastroenterol* **2014**, 14, 207.
17. Marinkelle, C. J.; German, E., Mansonelliasis in the Comiasria del Vaupes of Colombia. *Trop Geogr Med* **1970**, 22
18. Raccurt, C. P., et al., Epidemiologic survey of *Mansonella ozzardi* in Corail, Haiti. *Am J Trop Med Hyg* **2014**, 90 (6), 1167-9.
19. Veggiani Aybar, C. A., et al., *Mansonella ozzardi* in Neotropical region of Argentina: Prevalence through time (1986-2010). *Acta Trop* **2016**, 153, 1-6.
20. Medeiros, J. F., et al., *Mansonella ozzardi* in Brazil: prevalence of infection in riverine communities in the Purus region, in the state of Amazonas. *Mem Inst Oswaldo Cruz* **2009**, 104 (1), 74-80.
21. Raccurt, C. P., et al., Mansonelliasis, a neglected parasitic disease in Haiti. *Mem Inst Oswaldo Cruz* **2014**, 109 (6), 709-11.
22. Nathan, M. B., et al., Filariasis in Amerindians of western Guyana with observations on transmission of *Mansonella ozzardi* by a *Simulium* species of the amazonicum group. *Tropenmedizin und Parasitologie* **1982**, 33 (4), 219-22.
23. Krolewiecki, A. J., et al., Ivermectin-related adverse clinical events in patients treated for *Mansonella ozzardi* infections. *Rev Argent Microbiol* **2011**, 43 (1), 48-50.
24. Basano Sde, A., et al., Sustained clearance of *Mansonella ozzardi* infection after treatment with ivermectin in the Brazilian Amazon. *Am J Trop Med Hyg* **2014**, 90 (6), 1170-5.
25. Nutman, T. B., et al., Ivermectin in the successful treatment of a patient with *Mansonella ozzardi* infection. *J Infect Dis* **1987**, 156 (4), 662-5.
26. Nutman, T. B., et al., Ivermectin in the successful treatment of a patient with *Mansonella ozzardi* infection. *Dis* **1997**, 156.
27. Bartholomew, C. F., et al., The failure of diethylcarbamazine in the treatment of *Mansonella ozzardi* infections. *Trans R Soc Trop Med Hyg* **1978**, 72 (4), 423-4.
28. Bassene, H., et al., High Prevalence of *Mansonella perstans* Filariasis in Rural Senegal. *Am J Trop Med Hyg* **2015**, 93 (3), 601-6.
29. Baird, J. K., et al., Nodules in the conjunctiva, bung-eye, and bulge-eye in Africa caused by *Mansonella perstans*. *The American journal of tropical medicine and hygiene* **1988**, 38 (3), 553-7.
30. Wahlgren, M.; Frolov, I., Treatment of *Dipetalonema perstans* infections with mebendazole. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1983**, 77 (3), 422-3.
31. Gardon, J., et al., Efficacy of repeated doses of ivermectin against *Mansonella perstans*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2002**, 96 (3), 325-6.
32. Van den Enden, E., et al., *Mansonella perstans* filariasis: failure of albendazole treatment. *Annales de la Societe belge de medecine tropicale* **1992**, 72 (3), 215-8.
33. Gehringer, C., et al., Molecular evidence of *Wolbachia* endosymbiosis in *Mansonella perstans* in Gabon, Central Africa. *J Infect Dis* **2014**, 210 (10), 1633-8.
34. Keiser, P. B., et al., Molecular identification of *Wolbachia* from the filarial nematode *Mansonella perstans*. *Mol Biochem Parasitol* **2008**, 160 (2), 123-8.

35. Grobusch, M. P., et al., No evidence of Wolbachia endosymbiosis with *Loa loa* and *Mansonella perstans*. *Parasitol Res* **2003**, 90 (5), 405-8.
36. Coulibaly, Y. I., et al., A randomized trial of doxycycline for *Mansonella perstans* infection. *N Engl J Med* **2009**, 361 (15), 1448-58.
37. Asgeirsson, H., et al., Successful treatment of 2 imported cases of *Mansonella perstans* infection. *PLoS Negl Trop Dis* **2017**, 11 (5), e0005452.
38. Keiser, P. B., et al., Clinical characteristics of post-treatment reactions to ivermectin/albendazole for *Wuchereria bancrofti* in a region co-endemic for *Mansonella perstans*. *The American journal of tropical medicine and hygiene* **2003**, 69 (3), 331-5.
39. Okelo, G. B., et al., *Mansonella streptocerca* in the Central African Republic. *Trans R Soc Trop Med Hyg* **1988**, 82 (3), 464.
40. Fischer, P., et al., Occurrence and diagnosis of *Mansonella streptocerca* in Uganda. *Acta Trop* **1997**, 63 (1), 43-55.
41. Fischer, P., et al., Detection of the filarial parasite *Mansonella streptocerca* in skin biopsies by a nested polymerase chain reaction-based assay. *Am J Trop Med Hyg* **1998**, 58 (6), 816-20.
42. Orihel, T. C.; J., The tail of the *Mansonella streptocerca* microfilaria. *Am Med Hyg* **1984**, 33 (6), 1278.
43. Fischer, P., et al., Long-term suppression of *Mansonella streptocerca* microfilariae after treatment with ivermectin. *The Journal of infectious diseases* **1999**, 180 (4), 1403-5.
44. Fischer, P., et al., Treatment of human *Mansonella streptocerca* infection with ivermectin. *Trop Med Int Health* **1997**, 2 (2), 191-9.
45. Polderman, A. M., et al., Oesophagostomiasis, a common infection of man in northern Togo and Ghana. *The American journal of tropical medicine and hygiene* **1991**, 44 (3), 336-44.
46. Storey, P. A., et al., Natural progression of *Oesophagostomum bifurcum* pathology and infection in a rural community of northern Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2001**, 95 (3), 295-9.
47. Bogers, J. J., et al., Human oesophagostomiasis: a histomorphometric study of 13 new cases in northern Ghana. *Virchows Archiv : an international journal of pathology* **2001**, 439 (1), 21-6.
48. Ziem, J. B., et al., Distribution and clustering of *Oesophagostomum bifurcum* and hookworm infections in northern Ghana. *Parasitology* **2006**, 132 (Pt 4), 525-34.
49. Kepel, H. P., et al., Egg production of *Oesophagostomum bifurcum*, a locally common parasite of humans in Togo. *Am Med Hyg* **1992**, 46, 469-472.
50. Storey, P. A., et al., Intraobserver and interobserver variation of ultrasound diagnosis of *Oesophagostomum bifurcum* colon lesions. *The American journal of tropical medicine and hygiene* **2002**, 67 (6), 680-3.
51. Verweij, J. J., et al., Determining the prevalence of *Oesophagostomum bifurcum* and *Necator americanus* infections using specific PCR amplification of DNA from faecal samples. *Trop Med Int Health* **2001**, 6 (9), 726-31.
52. Ziem, J. B., et al., The short-term impact of albendazole treatment on *Oesophagostomum bifurcum* and hookworm infections in northern Ghana. *Annals of tropical medicine and parasitology* **2004**, 98 (4), 385-90.
53. Bradley, M., Rate of expulsion of *Necator americanus* and the false hookworm *Ternidens deminutus* Railliet and Henry 1909 (Nematoda) from humans following albendazole treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1990**, 84 (5), 720.
54. Kilala, C. P., *Ternidens deminutus* infecting man in Southern Tanzania. *East Afr Med J* **1971**, 48 (11), 636-45.
55. Hemsrichart, V., *Ternidens deminutus* infection: first pathological report of a human case in Asia. *J Med Assoc Thai* **2005**, 88 (8), 1140-3.
56. Goldsmid, J. M.; Saunders, C. R., Preliminary trial using pyrantal pamoate for the treatment of human infections with *Teernidens deminutus*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1972**, 66 (2), 375-6.
57. Goldsmid, J. M., Thiabendazole in the treatment of human infections with *Ternidens deminutus* (Nematoda). *S Afr Med J* **1972**, 46 (30), 1046-7.

27. Aberrant Nematode Infections

Many nematodes are zoonotic and only occasionally infect humans. Aberrant infections cannot mature into adult parasites in the human host. *Cutaneous larva migrans* (CLM) and *visceral larva migrans* (VLM) are two diseases caused by this kind of parasite. The nematodes causing CLM and VLM and the clinical manifestations of these diseases are listed in Tables 27.1 and 27.2. Although the number of nematode species resulting in aberrant infections is large, this chapter emphasizes only the most important ones, as defined by the seriousness of the diseases they induce.

Cutaneous Larva Migrans

CLM (Table 27.1) has a worldwide distribution. It is caused by larvae of the dog and cat hookworms *Ancylostoma braziliense*, (an-sə-'lās-tə-mə) and *Uncinaria stenocephala* (un-sin-NAR-EE-ah\ste-NO-seh-FAAL-ah\, \ən-sə-'nar-ē-ə\ste-nō-sə'fāl-ə). *A. braziliense* and *U. stenocephala* complete their life cycle in these hosts, similar to the way human hookworms behave. Zoonotic transmission from the dog hookworm *A. caninum* also occurs in humans, but disease from this parasite usually causes eosinophilic enteritis rather than CLM (see The Hookworms). Other less common nematodes may also be responsible for CLM, including a raccoon-transmitted *Strongyloides procyonis* (prō-sē-'ä-nəs) that results in “duck hunter’s itch.” The L3 larvae (Fig. 27.1) of *A. braziliense* survive in sandy, moist soils for several days. These larvae are especially common on beaches in Southeast Asia and the Caribbean, where dogs and cats can wander the beaches and freely defecate. In the United States, CLM occurs occasionally along the Gulf and Atlantic coasts of Florida and the beaches of the Carolinas. In the human host, infection begins when the L3 larvae penetrate unbroken skin but either fail to receive the proper

environmental cues or lack the necessary collagenase for deeper penetration. Rather than going further in their life cycle, instead, they migrate laterally in the deeper layers of the epidermis (Fig. 27.2) and can survive there for about 10 days.^{1,2}

CLM is characterized by an intense inflammatory reaction, associated with itching in the affected areas, which develops within days after the larvae enter the dermis. The secretions of the larvae, which consist of hydrolytic enzymes, provoke this inflammatory reaction. The serpiginous lesions known as “creeping eruption” are evident after an incubation period of one week. Secondary bacterial infections caused by scratching are common. In one review of CLM patients seen at a travelers’ clinic, 39% of the lesions appeared on the feet, 18% on the buttocks, 16% on the abdomen, and the remainder on the lower legs, arms, and face.³ In another review of patients with CLM seen at a travelers’ clinic in Munich, 73% of the lesions were found on the lower extremities, 13% in the buttocks and anogenital region, and 7% in the trunk and upper extremities.⁴

Diagnosis (see Clinical Appendix)

Some patients with CLM manifest eosinophilia or elevated IgE, but laboratory findings generally play little or no role in establishing a diagnosis of CLM.⁴ Physical findings, such



Figure 27.1. Third stage larva of *Ancylostoma braziliense*. Photo E. Gravé.

as the visualization of serpiginous lesions and dermoscopic findings of translucent brown areas with red-dotted vessels support the diagnosis.⁵

Treatment (see Clinical Appendix)

The treatment of choice for CLM is oral anti-helminthic therapy. A single dose of ivermectin (200 µg/kg once, sometimes repeated) is considered more effective than a single dose of albendazole, but repeated treatments of albendazole (400 mg daily for 5–7 days) are comparable. Some investigators recommend a 3-day course of albendazole (400 mg daily). Topical thiabendazole in a concentration of 10% to 15% 3 times daily for 5–7 days is an alternative treatment.⁶ Cryotherapy with liquid nitrogen can cause blistering and ulceration of the skin, so many experts recommend that this should not be performed.⁷

Visceral Larva Migrans

Toxocara canis

(Johnston 1916)

Toxocara cati

(Brumpt 1927)

Pronunciation: \tāk-sə-ˈkar-ə\

Beside *Enterobius vermicularis* (pinworm), *Toxocara* (TOK-so-ka-rah) may be the most common helminth infection in the United States. Seroprevalence in many areas of the United States and Mexico often exceeds 20%, especially among some African American populations living in poverty.^{8,9} The true burden of disease resulting from this large number of infections is poorly characterized.⁸ VLM and *ocular larva migrans* (OLM) are typically caused by *Toxocara canis* and *T. cati*. Aberrant migration of larvae through the viscera (Table 27.2) results in a far more



Figure 27.2. “Creeping eruption” on the foot of a patient who stepped on an infective larva of *A. braziliense*. Courtesy G. Zalar.

serious condition than CLM.

Human infection with *Toxocara* spp. was first described by Helenor Campbell Wilder in 1950 when he discovered a larva within a retinal granuloma of a child.¹⁰ In 1952, Paul Beaver and colleagues reported on a series of children who had high circulating eosinophilia and suffered severe multisystem disease caused by *T. canis* and *T. cati* larvae.¹¹ *T. canis* and *T. cati* have a life cycle in their respective hosts resembling *Ascaris* in humans. *Toxocara* adults (Fig. 27.3) are smaller than those of *Ascaris*, but are similar to them regarding nutritional requirements and physiologic behavior.¹² Since the discovery of VLM and OLM, some investigators have found that a significant percentage of children may suffer a so-called “covert” form of the disease in which patients do not have the full-blown syndrome of VLM, but more subtle manifestations including pulmonary dysfunction, or cognitive deficits leading to developmental delays.^{13–15}

In humans, the infection begins with the

Table 27.1. Cutaneous larva migrans: clinical manifestations

Organism	Predominant location in body	Major pathologic consequences	Diagnosis
<i>Ancylostoma braziliense</i>	Skin	Urticaria, serpiginous lesion	Biopsy
<i>Uncinaria stenocephala</i>	Skin	Urticaria, serpiginous lesion	Biopsy
<i>Strongyloides procyonis</i>	Skin “Duck-hunter’s itch”	Urticaria	Biopsy
<i>Dirofilaria conjunctivae</i>	Palpebral conjunctivae	Abscess formation subcutaneous tissues	Biopsy
<i>Dirofilaria repens</i>	Subcutaneous tissues	Fibrotic, painless nodule formation	Biopsy
<i>Anatrichosoma cutaneum</i>	Subcutaneous tissues	Serpiginous lesions	Biopsy
<i>Rhabditis niellyi</i>	Skin	Papule, urticaria	Biopsy
<i>Lagochilascaris minor</i>	Subcutaneous tissues around head and neck	Abscess formation	Biopsy
<i>Gnathostoma spinigerum</i>	Subcutaneous tissues	Abscess formation	Biopsy, ELISA
<i>Thelazia callipaeda</i>	Conjunctival sac, corneal conjunctiva	Paralysis of lower eyelid muscles, ectropion, fibrotic scarring	Ophthalmoscopic examination of conjunctiva

ELISA: enzyme-linked immunosorbent assay.

Table 27.2. Visceral larva migrans: clinical manifestations

Organism	Predominant location in body	Major pathologic consequences	Diagnosis
<i>Toxocara cati</i>	Viscera, eye	Blindness	ELISA
<i>Toxocara canis</i>	Viscera, eye	Blindness	ELISA
<i>Baylisascaris procyonis</i>	Meninges	Meningoencephalitis	CSF examination
<i>Angiostrongylus cantonensis</i>	Meninges	Meningoencephalitis	CSF examination
<i>Angiostrongylus costaricensis</i>	Mesenteric arterioles	Peritonitis	Biopsy
<i>Anisakis spp.</i>	Stomach wall	Granuloma	Biopsy
<i>Phocanema spp.</i>	Stomach wall	Granuloma	Biopsy
<i>Terranova spp.</i>	Stomach wall	Granuloma	Biopsy
<i>Oesophagostomum spp.</i>	Small and large intestinal wall	Granuloma	Biopsy
<i>Gnathostoma spinigerum</i>	Striated muscles, subcutaneous tissues, brain, small intestinal wall	Abscess, meningoencephalitis	Biopsy, ELISA
<i>Dirofilaria immitis</i>	Lung, heart	Granuloma in lung	Biopsy

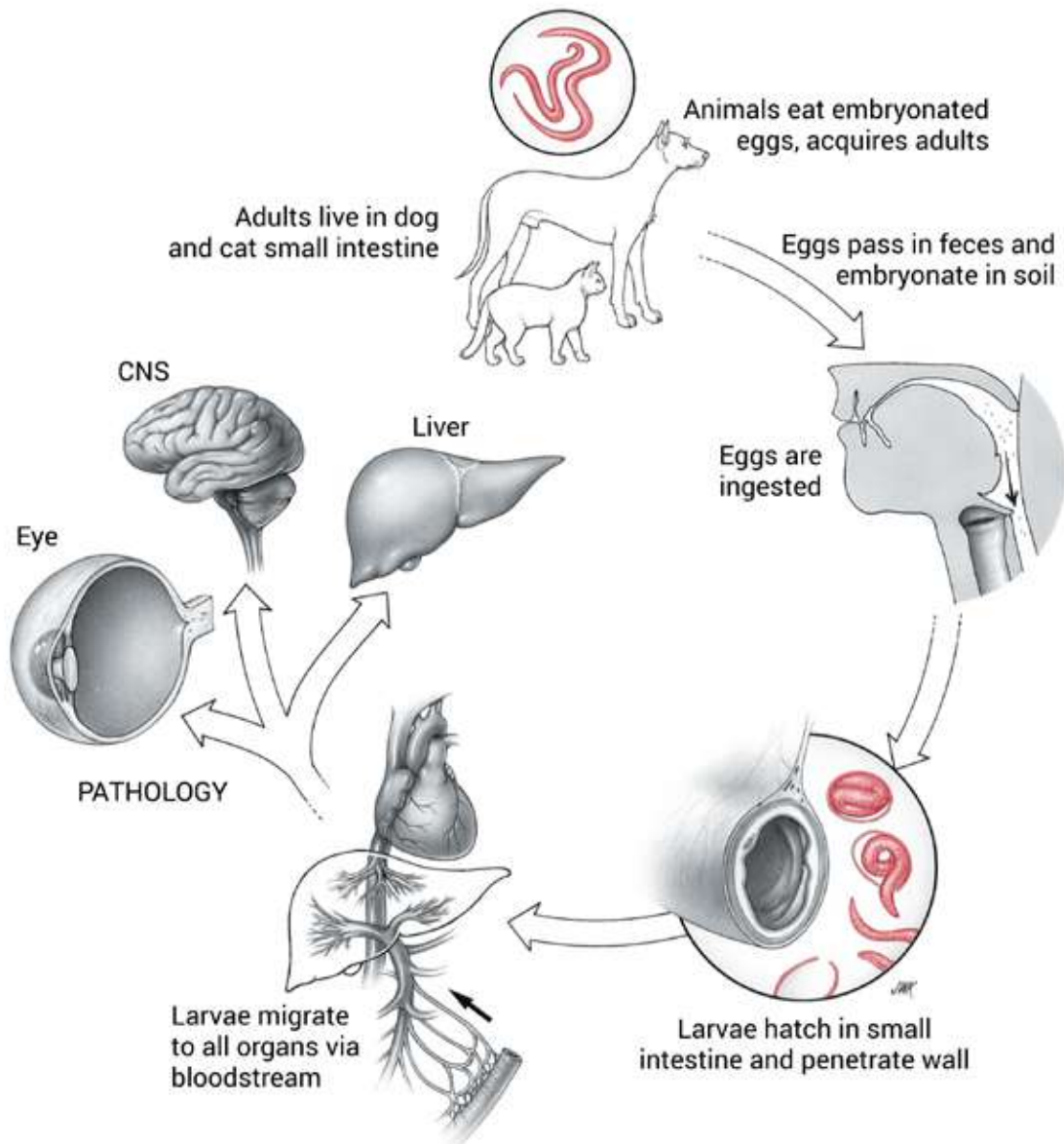
ELISA: enzyme-linked immunosorbent assay; RIA: radioimmunoassay; CSF: cerebrospinal fluid.

ingestion of embryonated *Toxocara* eggs (Fig. 27.4). This commonly occurs when children are playing in contaminated sandboxes and playgrounds. This situation is especially common in poor neighborhoods where stray dogs and cats are widespread. Pathology results when larvae hatch in the small intestine, penetrate the mesenteric blood vessels, and migrate throughout the body, invading all organs. There is controversy as to whether these are L2 or L3 larvae.

The degree of host damage varies with the tissues invaded: the liver, lungs, and CNS (Fig. 27.5), including the eyes, are the organ

systems most seriously affected. Ultimately, the larvae die after weeks to months of migration, followed by marked delayed-type and immediate-type hypersensitivity responses. These inflammatory responses manifest as eosinophilic granulomas. The immediate hypersensitivity responses to dead and dying larvae in the viscera, including the lungs, liver, and brain, result in VLM. In the eye, the larvae causing OLM can affect the retina, where the larval tracks and granulomas are sometimes mistaken for retinoblastoma (Fig. 27.6.). As a result of this similarity, unnecessary enucleation has been carried out in some cases. In many cases, the granuloma was

Toxocara canis and *Toxocara cati*



responsible for losing sight.^{16, 17}

Epidemiologic evidence suggested that ocular disease tended to occur without systemic involvement and *vice versa*. This led to the proposal that the two manifestations of this infection be reclassified as OLM and VLM.¹⁸ There may be strains of *T. canis* with specific tropisms. Alternatively, VLM may reflect the consequences of a host inflammatory response to repeated waves of migrating larvae through the viscera. In contrast, OLM occurs in individuals who have not been previously sensitized.¹²

VLM and Covert Toxocariasis

VLM is mainly a disease of young children (<5 years of age).^{19, 20} VLM presents with



Figure 27.3. Adults of *Toxocara canis*. The female is 9 cm, the male is 6 cm.



Figure 27.4. Embryonated egg of *Toxocara canis*.

fever, hepatosplenomegaly, lower respiratory symptoms (particular bronchospasm, resembling asthma), circulating eosinophilia (sometimes approaching 70%), and hypergammaglobulinemia of IgM and IgG classes. Myocarditis, nephritis, and involvement of the CNS have been described. CNS involvement can lead to seizures, neuropsychiatric symptoms, or an encephalopathy. *T. canis* can also be associated with eosinophilic meningoencephalitis.²¹

There has been an increasing appreciation that subtler clinical manifestations might also arise due to larval migrations. So-called “covert toxocariasis” ranges from asymptomatic infection to larvae migrating in specific target organs.²²⁻²⁴ Covert toxocariasis is felt to be due to chronic exposure.²⁵ In the lungs, larval migrations may result in asthma. *T. canis* has been suggested as an environmental risk factor for asthma among some inner-city populations.²⁶⁻²⁸ In the brain, *T. canis* has been implicated as one of the causes of “idiopathic” seizure disorders, and infection is significantly more prevalent in children with mental disabilities, including non-institutionalized children.^{14, 15, 29, 30} Toxocariasis has also been linked to functional intestinal disorders, arthritis, and skin rashes.^{23, 26, 31, 32} There is a concern that the cases of *Toxocara* spp. identified through serological testing may be associated with long-term cognitive consequences. Measurable reductions in scores on

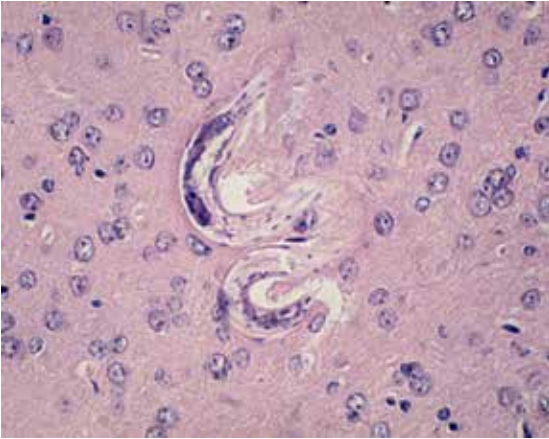


Figure 27.5. Larvae in brain of mouse experimentally infected with *T. canis*.

cognitive tests have been demonstrated when comparing children with serological evidence of *Toxocara* infection to those without.¹⁴ Longitudinal studies to confirm these findings are still needed, but concern has been raised that the impact of this disease goes far beyond the cases brought to medical attention by symptoms. The full clinical spectrum of disease of covert toxocariasis has yet to be explored.^{15,33}

OLM

Ocular larva migrans (OLM) usually occurs in older children (5–10 years old), and typically presents as unilateral vision impairment that is sometimes accompanied by strabismus.³¹ In temperate climates, such as in the UK, it is estimated that the prevalence of OLM may be as high as 9.7 cases per 100,000 persons.³⁴ The most serious consequence of the infection is invasion of the retina, leading to granuloma formation peripherally or in the posterior pole. These granulomas drag the retina and distort or detach the macula.³⁵ The degree of visual acuity impairment depends on the area involved, and blindness is common. OLM can also cause diffuse endophthalmitis or papillitis; secondary glaucoma can follow. In some cases, these lesions have been visualized on ophthalmic examination, and due to their visual similarities with reti-

noblastoma, there is the risk of unnecessary enucleation of the infected eye.³⁶⁻³⁹

Diagnosis (see Clinical Appendix)

Any pediatric-age patient with an unexplained febrile illness and eosinophilia should be suspected of having VLM. Hepatosplenomegaly and evidence of multisystem disease with a history of pica make diagnosing VLM more likely. Similarly, OLM should be suspected in any child with unilateral vision loss and strabismus. Diagnostic tests for VLM are primarily immunological.⁴⁰

The precipitin test is subject to cross-reactions with common antigens of the larvae and blood group substance A. The recommended test for toxocariasis detects IgG against excretory-secretory antigens derived from *Toxocara* larvae. A *Toxocara* ELISA is available through the Center for Disease Control and Prevention (CDC), and is estimated to be 78% sensitive and 92% specific when using the appropriate cutoff.^{41, 42} Newer diagnostics are also under development, including those using recombinant antigens.^{43, 44} Stool examination of infected individuals is not helpful, as these aberrant nematodes do not complete their life cycle in humans, so no eggs are produced.



Figure 27.6. Granuloma in retina of patient with OLM. The lesion consists largely of eosinophils.

Other indicators include hypergammaglobulinemia and an elevated isohemagglutinin titer. A constellation of clinical disease, a history of pica, eosinophilia, and positive serology strongly point to the diagnosis. Liver biopsy may reveal a granuloma surrounding a larva, but a successful diagnosis using this approach is fortuitous at best and not recommended. OLM is diagnosed primarily based on clinical criteria during an ophthalmologic examination. The immunodiagnostic tests used for VLM are not as reliable for OLM. In one study, only 45% of patients with clinically diagnosed OLM had titers high enough to be classified as positive.⁴⁵

Treatment (see Clinical Appendix)

While there is limited reliable evidence-based guidance about treatment, *T. canis* and *T. cati* can be treated with albendazole 400mg oral 2x/day for 5 days (adult and pediatric dose).³² The other commonly used drug, mebendazole, is poorly absorbed outside the gastrointestinal (GI) tract. Some success has been reported in patients who ingest 1g or more of this agent for a 21-day course.⁴⁶ Symptomatic treatment, including the administration of corticosteroids, has helped suppress intense manifestations of the infection. OLM is treated by surgery (vitrectomy), antihelminthic chemotherapy, and/or corticosteroids.⁴⁷⁻⁵⁰ In the case of ocular involvement with active ocular inflammation, the role of antihelminthic therapy is unclear, owing to the lack of knowledge regarding the intra-ocular pharmacokinetics, pharmacodynamics, and the impact of therapy on outcomes.

The *Toxocara* group of parasites is common in young pets. For example, young puppies often harbor these worms, since the infection can be congenitally acquired. Having a litter of puppies in the home has been identified as a significant risk factor.⁴⁷ While *Toxocara* spp. are able to cause death in puppies, death

is rare in human infection. Children with pica are at risk of ingesting embryonated eggs from soil. Adult patients who have been institutionalized for mental retardation are also at risk.⁴⁸ Treatment of dogs and control of their feces are major control measures for this disease. Toxocariasis is an understudied and underreported disease. Through scattered serological surveys, there is increasing evidence that it is one of the most common helminthic diseases in temperate climates of North America and Europe, including some large inner-city urban areas, as well as in Brazil and elsewhere.^{8, 51-55}

Baylisascaris procyonis

(Sprent, 1968)

Pronunciation: \bā-lis-as-kə-rəs\pro-sī-ən-nəs\

Baylisascaris procyonis (\BAY-lis-as-KARIS\pro-SCI-on-is\), the raccoon ascarid, causes VLM and neural larva migrans in humans when they accidentally ingest embryonated eggs that are shed in sylvatic environs, as well as in peri-domestic environs, in which suburban dwellings permit accessibility to raccoons, such as attics and rain gutters.^{40, 42, 45} Although it is rare, the pathological

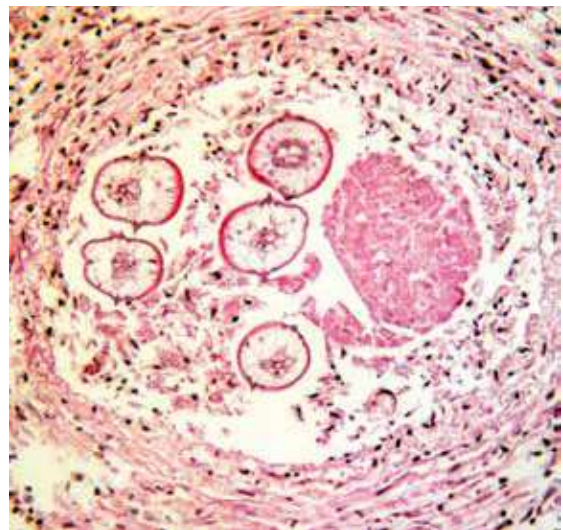


Figure 27.7. Larvae of *B. procyonis* in brain of child who died of VLM.

consequences of infection with the larva of *B. procyonis* is generally more severe than that caused by *T. canis*. Neural larva migrans can result in neuro-devastation, even with antihelminthic chemotherapy and steroids.⁵⁶ The large, developing larvae (up to 2 mm.) cause considerable mechanical damage.⁵⁶ Larvae migrating through brain tissue can result in eosinophilic meningitis associated with high mortality (Fig. 27.7). Neural larva migrans is associated with ocular disease characterized by retinitis and multiple choroidal infiltrates.⁵⁷

Since antibodies to *B. procyonis* antigens do not cross-react with *T. canis*, the excretory and secretory products of *B. procyonis* have been examined as a possible species-specific immunodiagnostic reagent.⁵⁸ Although a serological test has been developed for *B. procyonis* with high sensitivity and specificity, many human cases have been diagnosed at autopsy. There are a limited number of cases diagnosed to date, so there is little overall experience with antihelminthic therapy for baylisascariasis, but good outcomes have been reported with high dose albendazole 25–50 mg/kg/day in divided doses started promptly and continued for 10–20 days with concomitant steroids.^{59–61}

Ophidascaris robertsi

The L3 larva of an Australian ascarid parasite of carpet pythons, *Ophidascaris robertsi*, was the cause of a single case of VLM.⁶² The patient was a 64-year-old woman who was immunosuppressed. She was treated with ivermectin and made an uneventful recovery. Her house was within walking distance from a lake whose shoreline was frequented by carpet pythons. A fine example of : “*nature abhors a vacuum*”.

Angiostrongylus cantonensis

(Chen 1935)

Pronunciation: \an-jē-ə-'strän-jə-ləs\\kan-tən-ən-səs\\

Angiostrongylus costaricensis

(Morera and Cespedes 1971)

Pronunciation: \an-jē-ə-'strän-jə-ləs\\kò-stə-'rē-kən- səs\\

Angiostrongylus cantonensis (AN-jee-o-STRON-jah-lus\\KAN-ton-en-SIS\\) occurs in Southeast Asia, the Philippine Islands, Taiwan, and North and South Pacific islands, including Hawaii. Human infection was first described in Taiwan when the parasite's larvae were isolated from a child's CSF. Infected rodents, but not human cases, have also been reported from East Africa. A survey of wharf rats in New Orleans revealed a high infection rate, although none had been detected several years before that study.⁵³ *A. cantonensis* is present in all the main Hawaiian Islands, and the eastern portion of the Big Island of Hawaii is the epicenter for this infection, paralleling the introduction of the semi-slug *Parmarion martensi*.⁶³ Acquisition of human infection depends upon the locale and the nutritional habits of the specific population. A case in Hawaii resulted from ingesting a raw slug for a traditional medicinal purpose. In Tahiti, epidemics have resulted from eating raw freshwater prawns, and elsewhere, other invertebrates (e.g., planarians) either consumed directly or in various leafy greens used in salads.

Rats are the definitive host for *Angiostrongylus* spp.; several species of wild rats, including the common wharf rat, *Rattus norvegicus*. These worms live and lay eggs in the

rodent's pulmonary arteries. The larvae hatch and move through the capillaries in the lung and then penetrate the alveolar spaces. From the alveolar spaces, the immature parasites migrate up the respiratory tree, are swallowed, and ultimately pass out of the rat in feces.⁶⁴ *A. cantonensis* larvae incubate in the soil before being consumed by various mollusks and crustaceans.⁶⁵⁻⁶⁸ Humans are infected when they accidentally consume these slugs, or the larval stages of *A. cantonensis* present in uncooked snails, slugs, or raw vegetables contaminated with secretions from these invertebrates. *A. costaricensis* (\AN-jee-o-STRON-jah-lus\\KOS-tar-EE-sen-sis\) larvae are generally restricted to infecting only one invertebrate host, the slug. In most cases, the specific slug is *Vaginulus plebius*.

Most cases of human infection with *Angiostrongylus* spp. result in the failure of the worm to complete its life cycle. Once in a human host, the larvae often migrate to the brain or rarely to the lungs, as observed in infants and small children. These nematodes die surrounded by an eosinophil-rich inflammatory infiltrate.⁶⁹ The pattern of clinical presentation varies with each species of *Angiostrongylus*. *A. cantonensis* L3 larvae usually migrate to the meningeal capillaries, where their death triggers inflammation resulting in eosinophilic meningoencephalitis. Part of the pathology can involve vascular thrombosis and aneurysms. Painful paresthesias, headache, and fever are described as features seen in the clinical presentation of this infection.⁷⁰ Vomiting is reported in most cases, as are migrating painful paresthesias.⁷¹ Painful paresthesias often persist for weeks to months, along with headaches. Although eosinophilic meningitis is characteristic on CSF examination, focal lesions are usually not present on head CT or MRI. This can be useful in distinguishing the disease from other etiologies, such as *Gnathostoma spinigerum* and neurocysticercosis.⁷²

A. cantonensis is the most common cause of eosinophilic meningitis outside of the developed world and should be considered in the differential of travelers returning from endemic areas.⁶⁹ The natural course of the disease without treatment is resolution of symptoms in 1–2 weeks. Disease duration and severity may worsen in children, and some individuals may have longer durations of illness despite treatment. In contrast to the clinical picture seen with *A. cantonensis*, *A. costaricensis* causes abdominal angiostrongyliasis. Patients may present with abdominal pain, fever, and peripheral eosinophilia, but since *A. costaricensis* does not migrate to the brain, this organism does not cause eosinophilic meningitis.⁷³⁻⁷⁵

Diagnosis of *Angiostrongylus* spp. is based on clinical presentation and PCR, as serological testing is not widely available. Confirmation of suspected cases has been achieved using post-treatment serological testing in concert with research laboratories.⁷¹ PCR testing is available and can be performed on CSF, but it lacks high sensitivity.⁷⁶

With the self-limiting nature of this disease, treatment often focuses on symptoms rather than on antihelmintic therapy. Although animal models have supported antihelmintic therapy trials, efficacy in human infection has not been demonstrated, and there are reports that antihelmintic therapy may lead to more serious disease.⁷⁷⁻⁷⁹ When these drugs are given, some experts recommend concurrent use of corticosteroids. Corticosteroids have been shown to reduce symptoms (primarily headaches) and thus are often used. In addition, serial lumbar punctures and analgesics are often part of the approach to managing these patients.

Widespread education about the proper cooking of food and vegetable washing, as well as the control of mollusks and planarians in

vegetable gardens, can reduce the incidence of infections.⁵²

Gnathostoma spinigerum

(Owen 1838)

Pronunciation: \nə-'thäs-tə-mə\\spi-ni-gerəm\

Gnathostoma spinigerum (nah-THOS-tah-mah\\SPI-ni-G'E-rum\)) is a nematode parasite of various mammals, including cats, dogs, and the mongoose. The intermediate hosts include 1. copepods in the genus *Cyclops*, and 2. snakes, frogs, fish, and birds. Gnathostomiasis in humans is prevalent throughout Mexico, Thailand, and Asia. In Mexico, infections are commonly acquired in and around Acapulco.^{56, 61, 80}

Female adults are 25–54 mm in length, while males measure 11–25 mm. The mature worms live coiled in the wall of the stomach of their definitive hosts. Eggs pass in feces and hatch in water, releasing larvae ingested by macroinvertebrate crustaceans. Host vertebrates eat infected crustaceans, and the infective stage for humans then develops within them. The larvae invade the small intestinal tissue when humans eat these infected vertebrates. The worms leave the intestine and migrate into the deep tissues.⁸¹⁻⁸³

Although the infection is asymptomatic in most cases: skin manifestations can include CLM, panniculitis, and subcutaneous swellings. Invasion of the CNS can occur with peripheral eosinophilia, eosinophilic meningitis with radicular pain, and paresthesias due to larval migration. *G. spinigerum* can also invade the eye, resulting in an intraocular form of this disease.⁸⁴

Previously, the diagnosis of gnathostomiasis was limited to an appropriate travel history, clinical presentation, and peripheral eosinophilia, but diagnostic tests have become

available. ELISA and Western Blot aid in diagnosis, but these are only available at laboratories outside of the United States, and definitive diagnosis with parasite isolation is often impossible.⁸⁵

Although this disease can be self-limited, therapy often involves a 21-day oral course of albendazole at a dose of 400mg once daily.⁸⁶ Ivermectin was effective in several trials and may be considered an alternate agent. It remains unclear whether antihelmintic therapy's role in ocular and neurological disease may worsen symptoms. In some cases, surgical removal is required.⁵⁶ Corticosteroids have been used to treat patients with CNS manifestations. Relapses have been reported with either therapy.⁷¹

Anisakiasis and Related Illnesses

Anisakiasis in humans is caused by a number of species of nematodes belonging to the genera *Anisakis* (\an-ee-SAY-kiss\\a-nə-'sā-kəs\), *Phocanema*, *Terranova* (\TEAR-ah-now-vah\\ter-ə-'nō-və\), and *Contracaecum*. They infect sea mammals, particularly dolphins, whales, sea lions, and seals.⁸⁷ In these hosts, adults live in the lumen of the stomach. Anisakid L2 larvae infect several crustacean species. L3 larvae infect a wide variety of bottom-feeding fish.⁸⁸

The adult worms of marine mammal anisakids embed in the gastric mucosa and pass unembryonated eggs out into the environment in the feces. The eggs embryonate, and larvae mature inside the eggs until free-swimming larvae are released. Crustaceans ingest these larvae. They mature into the stage infective for fish and squid; paratenic hosts that transport the L3 larvae to their mammalian hosts. After being ingested, rather than staying in the fish or squid intestine, larvae migrate to the peritoneal cavity, growing up to 3 cm long. Upon the death of the cold-blooded vertebrate host, larvae migrate to their muscles,

where they may be ingested by warm-blooded marine predator/scavengers, and develop into adult worms. After mating and then embedding into the mucosa, adult female worms begin laying eggs. At this point, the life cycle is complete.⁸⁹

Raw or undercooked saltwater fish, often in the form of sushi or sashimi, has become a popular style of cuisine throughout the world.^{57, 58} When an infected piece of raw fish is eaten, the parasites in the muscle tissue are released by the enzymes in the stomach or more rarely, into the small intestine. The release of parasite hydrolytic enzymes facilitates tissue invasion.^{90, 91} Infected individuals often present with abdominal pain that can be severe and can be confused with the symptoms of an acute gastric ulcer.⁹² From ingestion of larvae to onset of symptoms usually lasts minutes to hours. The presentation can include nausea, vomiting, abdominal distension, mild fever, and diarrhea with blood and mucous in the stool.⁹³ If larvae can penetrate the mucosa, angioedema, urticarial, and allergic symptoms can dominate the clinical presentation.⁹⁴

All species of anisakid worms die within a few days in humans. Dead parasites provoke

an eosinophilic granulomatous infiltration. Initially, infection may be asymptomatic, but soon thereafter, vague upper abdominal pain may develop.

Definitive diagnosis and treatment are made by removal of the parasite. Sometimes, the worms are expelled through coughing or vomiting, but at other times, endoscopic removal or surgery for extra-intestinal manifestations is necessary. Serologic tests using an antigen capture ELISA are available in some countries and have sensitivities reported near 100%.⁹⁵ Commercial PCR tests have been developed and are available.

Thorough cooking or freezing of seafood before ingestion can prevent infection by anisakid nematodes. Most sushi restaurants in the United States and elsewhere now inspect pieces of raw fish carefully before serving them, and the FDA under Food Code 101 requires freezing before serving raw fish. Flash freezing in liquid nitrogen has become a popular way of processing fish destined for sushi restaurants. After a peak in the 1980s, the incidence of anisakiasis in Europe and North America due to the consumption of raw fish has been reduced to a few sporadic cases annually.⁹⁶

References

1. Sulica, V. I., et al., Histopathologic findings of cutaneous larva migrans. *Pathol* 346 **1988**, 15.
2. Bowman, D. D., et al., Hookworms of dogs and cats as agents of cutaneous larva migrans. *Trends Parasitol* **2010**, 26 (4), 162-7.
3. Blackwell, V.; Vega-Lopez, F., Cutaneous larva migrans: clinical features and management of 44 cases presenting in the returning traveller. *The British journal of dermatology* **2001**, 145 (3), 434-7.
4. Caumes, E., It's time to distinguish the sign 'creeping eruption' from the syndrome 'cutaneous larva migrans'. *Dermatology* **2006**, 213 (3), 179-81.
5. Aljasser, M. I., et al., Dermoscopy and near-infrared fluorescence imaging of cutaneous larva migrans. *Photodermatol Photoimmunol Photomed* **2013**, 29 (6), 337-8.
6. Albanese, G.; Venturi, C., Albendazole: a new drug for human parasitoses. *Dermatologic clinics* **2003**, 21 (2), 283-90.
7. Albanese, G., et al., Treatment of larva migrans cutanea (creeping eruption): a comparison between albendazole and traditional therapy. *Int J Dermatol* **2001**, 40 (1), 67-71.
8. Lee, R. M., et al., Toxocariasis in North America: a systematic review. *PLoS Negl Trop Dis* **2014**, 8 (8), e3116.

9. Won, K. Y., et al., National seroprevalence and risk factors for Zoonotic *Toxocara* spp. infection. *Am J Trop Med Hyg* **2008**, 79 (4), 552-7.
10. Wilder, H. C., Nematode endophthalmitis. *Trans Am Acad Ophthalmol Otolaryngol* **1950**, 55 99-104.
11. Beaver, P. C.; Snyder, C. H., Chronic eosinophilia due to visceral larva migrans. *Pediatrics* **1952**, 9 7-19.
12. Despommier, D., Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. *Clinical microbiology reviews* **2003**, 16 (2), 265-72.
13. Walsh, M. G.; Haseeb, M. A., Toxocariasis and lung function: relevance of a neglected infection in an urban landscape. *Acta Parasitol* **2014**, 59 (1), 126-31.
14. Walsh, M. G.; Haseeb, M. A., Reduced cognitive function in children with toxocariasis in a nationally representative sample of the United States. *Int J Parasitol* **2012**, 42 (13-14), 1159-63.
15. Hotez, P. J., Neglected infections of poverty in the United States and their effects on the brain. *JAMA Psychiatry* **2014**, 71 (10), 1099-100.
16. Despommier, D., Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. *Clin Microbiol Rev* **2003**, 16 (2), 265-72.
17. Ahn, S. J., et al., Ocular toxocariasis: clinical features, diagnosis, treatment, and prevention. *Asia Pac Allergy* **2014**, 4 (3), 134-41.
18. Glickman, L. I.; Schantz, P. M., Epidemiology and pathogenesis of zoonotic toxocariasis. *Epidemiol Rev* **1982**, 10, 143-148.
19. Kazacos, K. R., Visceral and ocular larva migrans. *Seminars in veterinary medicine and surgery (small animal)* **1991**, 6 (3), 227-35.
20. Worley, G., et al., *Toxocara canis* infection: clinical and epidemiological associations with seropositivity in kindergarten children. *J Infect Dis* **1984**, 149 (4), 591-7.
21. Xinou, E., et al., CT and MR imaging findings in cerebral toxocaral disease. *AJNR. American journal of neuroradiology* **2003**, 24 (4), 714-8.
22. Taylor, M. R., et al., The expanded spectrum of toxocaral disease. *Lancet (London, England)* **1988**, 1 (8587), 692-5.
23. Sharghi, N., et al., Toxocariasis: An occult cause of childhood asthma, seizures, and neuropsychological deficit? . *Sem Pediatr Infect Dis* **2000**, 11 257-60.
24. Nathwani, D., et al., Covert toxocariasis--a cause of recurrent abdominal pain in childhood. *The British journal of clinical practice* **1992**, 46 (4), 271.
25. Minvielle, M. C., et al., [Asthma and covert toxocariasis]. *Medicina (B Aires)* **1999**, 59 (3), 243-8.
26. Buijs, J., et al., *Toxocara* seroprevalence in 5-year-old elementary schoolchildren: relation with allergic asthma. *American journal of epidemiology* **1994**, 140 (9), 839-47.
27. Tariq, S. M., et al., Epidemiology of allergic disorders in early childhood. *Pediatric pulmonology. Supplement* **1997**, 16, 69.
28. Sharghi, N., et al., Environmental exposure to *Toxocara* as a possible risk factor for asthma: a clinic-based case-control study. *Clin Inf Dis* **2001**, 32 (7), E111-6.
29. Critchley, E. M., et al., Toxoplasma, Toxocara, and epilepsy. *Epilepsia* **1982**, 23 (3), 315-21.
30. Kaplan, M., et al., The frequency of *Toxocara* infection in mental retarded children. *Memorias do Instituto Oswaldo Cruz* **2004**, 99 (2), 121-5.
31. Konate, A., et al., [Toxocariasis and functional intestinal disorders. Presentation of 4 cases]. *Gastroenterologie clinique et biologique* **1996**, 20 (10), 909-11.
32. Dinning, W. J., et al., Toxocariasis: a practical approach to management of ocular disease. *Eye (London, England)* **1988**, 2 (Pt 5), 580-2.
33. Magnaval, J. F., et al., Therapy and Prevention for Human Toxocariasis. *Microorganisms* **2022**, 10 (2).
34. Good, B., et al., Ocular toxocariasis in schoolchildren. *Clin Inf Dis* **2004**, 39 (2), 173-8.
35. Small, K. W., et al., Surgical management of retinal traction caused by toxocariasis. *American journal of ophthalmology* **1989**, 108 (1), 10-4.
36. Ota, K. V., et al., Radiologic surveillance for retinoblastoma metastases unexpectedly showed disseminated toxocariasis in liver, lung, and spinal cord. *Canadian journal of ophthalmology. Journal canadien d'ophtalmologie* **2010**, 45 (2), 185-6.
37. Ota, K. V., et al., Toxocariasis mimicking liver, lung, and spinal cord metastases from

- retinoblastoma. *Pediatr Infect Dis J* **2009**, 28 (3), 252-4.
38. Lopez-Velez, R., et al., [Ocular toxocariasis or retinoblastoma?]. *Enferm Infecc Microbiol Clin* **1995**, 13 (4), 242-5.
 39. Van Nerom, P. R., et al., [Differential diagnosis between retinoblastoma and ocular toxocariasis]. *Journal francais d'ophtalmologie* **1987**, 10 (4), 279-82.
 40. Schantz, P. M., Toxocara larva migrans now. *Am J Trop Med Hyg* **1989**, 41 (3 Suppl), 21-34.
 41. Rubinsky-Elefant, G., et al., Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. *Ann Trop Med Parasitol* **2010**, 104 (1), 3-23.
 42. Fong, M. Y.; Lau, Y. L., Recombinant expression of the larval excretory-secretory antigen TES-120 of *Toxocara canis* in the methylotrophic yeast *Pichia pastoris*. *Parasitol Res* **2003**, 92, 173-6.
 43. Anderson, J. P., et al., Development of a Luminex Bead Based Assay for Diagnosis of Toxocariasis Using Recombinant Antigens Tc-CTL-1 and Tc-TES-26. *PLoS Negl Trop Dis* **2015**, 9 (10), e0004168.
 44. Zhan, B., et al., Identification of immunodominant antigens for the laboratory diagnosis of toxocariasis. *Trop Med Int Health* **2015**, 20 (12), 1787-96.
 45. Schantz, P. M., et al., Clinical, serologic, and epidemiologic characteristics of ocular toxocariasis. *Am J Trop Med Hyg* **1979**, 28 (1), 24-8.
 46. Hotez, P. J., et al., *Toxocara canis*. 1995.
 47. Marmor, M., et al., *Toxocara canis* infection of children: epidemiologic and neuropsychologic findings. *American journal of public health* **1987**, 77 (5), 554-9.
 48. Hummer, D., et al., Seroepidemiologic study of toxocariasis and strongyloidiasis in institutionalized mentally retarded adults. *Am Med Hyg* **1992**, 46, 278-281.
 49. Sturchler, D.; Schubarth, P., Thiabendazole v albendazole in treatment of toxocariasis a clinical trial. *Ann Trop Med Parasitol* **1989**, 83 473-478.
 50. Sturchler, D., et al., Thiabendazole vs. albendazole in treatment of toxocariasis: a clinical trial. *Ann Trop Med Parasitol* **1989**, 83 (5), 473-8.
 51. Gauthier, J. L., et al., Stealth parasites: the under appreciated burden of parasitic zoonoses in North America. 2001; p 1-21.
 52. Hotez, P. J., Reducing the global burden of human parasitic diseases. *Comp Parasitol* **2002**, 69, 140-45.
 53. Anaruma Filho, F.; Chieffi, P. P., Human toxocariasis: incidence among residents in the outskirts of Campinas, State of Sao Paulo, Brazil. *Rev Inst Med Trop Sao Paulo* **2003**, 45 (5), 293-294.
 54. Fialho, P. M.; Correa, C. R., A Systematic Review of Toxocariasis: A Neglected but High-Prevalence Disease in Brazil. *Am J Trop Med Hyg* **2016**.
 55. Borecka, A.; Klapiec, T., Epidemiology of human toxocariasis in Poland - A review of cases 1978-2009. *Ann Agric Environ Med* **2015**, 22 (1), 28-31.
 56. Kazacos, K. R., et al., *Baylisascaris* larva migrans. *VeMed Assoc* **1990**, 195, 894-903.
 57. Mets, M. B., et al., Eye findings of diffuse unilateral subacute neuroretinitis and multiple choroidal infiltrates associated with neural larva migrans due to *Baylisascaris procyonis*. *American journal of ophthalmology* **2003**, 135 (6), 888-90.
 58. Boyce, W. M., et al., Physicochemical characterization and monoclonal and polyclonal antibody recognition of *Baylisascaris procyonis* larval excretory-secretory antigens. *J Parasitol* **1989**, 75 (4), 540-8.
 59. Peters, J. M., et al., Good outcome with early empiric treatment of neural larva migrans due to *Baylisascaris procyonis*. *Pediatrics* **2012**, 129 (3), e806-11.
 60. Pai, P. J., et al., Full recovery from *Baylisascaris procyonis* eosinophilic meningitis. *Emerg Infect Dis* **2007**, 13 (6), 928-30.
 61. Gavin, P. J., et al., Neural larva migrans caused by the raccoon roundworm *Baylisascaris procyonis*. *The Pediatric infectious disease journal* **2002**, 21 (10), 971-5.
 62. Hossain, M. E., et al., Human Neural Larva Migrans Caused by *Ophidascaris robertsi* Ascarid. *Emerg Infect Dis* **2023**, 29 (9), 1900-1903.
 63. Jarvi, S. I., et al., High prevalence of *Angiostrongylus cantonensis* (rat lungworm) on eastern Hawai'i Island: A closer look at life cycle traits and patterns of infection in wild rats (*Rattus* spp.). *PLoS one* **2017**, 12 (12), e0189458.
 64. Mackerras, M. J.; Sandars, D. F., Lifehistory of the rat lung-worm and its migration through the

- brain of its host. *Nature* **1954**, 173 (4411), 956-7.
65. Qvarnstrom, Y., et al., PCR-based detection of *Angiostrongylus cantonensis* in tissue and mucus secretions from molluscan hosts. *Appl Environ Microbiol* **2007**, 73 (5), 1415-9.
66. Richards, C. S.; Merritt, J. W., Studies on *Angiostrongylus cantonensis* in molluscan intermediate hosts. *J Parasitol* **1967**, 53 (2), 382-8.
67. Wallace, G. D.; Rosen, L., Studies on eosinophilic meningitis. V. Molluscan hosts of *Angiostrongylus cantonensis* on Pacific Islands. *Am J Trop Med Hyg* **1969**, 18 (2), 206-16.
68. Drozd, J., et al., [Study of the morphology and larval development of *Angiostrongylus* (Parastrongylus) dujardini Drozd and Doby 1970, Nematoda: Metastrongyloidea. Infestation of the molluscan intermediary hosts]. *Annales de parasitologie humaine et comparee* **1971**, 46 (3), 265-76.
69. Slom, T. J., et al., An outbreak of eosinophilic meningitis caused by *Angiostrongylus cantonensis* in travelers returning from the Caribbean. *N Engl J Med* **2002**, 346 (9), 668-75.
70. Tseng, Y. T., et al., Clinical manifestations of eosinophilic meningitis caused by *Angiostrongylus cantonensis*: 18 years' experience in a medical center in southern Taiwan. *J Microbiol Immunol Infect* **2011**, 44 (5), 382-9.
71. Ramirez-Avila, L., et al., Eosinophilic meningitis due to *Angiostrongylus* and *Gnathostoma* species. *Clin Infect Dis* **2009**, 48 (3), 322-7.
72. Lo Re, V., 3rd; Gluckman, S. J., Eosinophilic meningitis. *Am J Med* **2003**, 114 (3), 217-23.
73. Morera, P., Life history and redescription of *Angiostrongylus costaricensis* Morera and Céspedes, 1971. *The American journal of tropical medicine and hygiene* **1973**, 22 (5), 613-21.
74. Morera, P.; Céspedes, R., *Angiostrongylus costaricensis* n. sp. (Nematoda: Metastrongyloidea), a new lungworm occurring in man in Costa Rica. 1971. *Rev Biol Trop* **2002**, 50 (2), 783-96.
75. Mota, E. M.; Lenzi, H. L., *Angiostrongylus costaricensis*: complete redescription of the migratory pathways based on experimental *Sigmodon hispidus* infection. *Mem Inst Oswaldo Cruz* **2005**, 100 (4), 407-20.
76. Eamsobhana, P., et al., Molecular diagnosis of eosinophilic meningitis due to *Angiostrongylus cantonensis* (Nematoda: Metastrongyloidea) by polymerase chain reaction-DNA sequencing of cerebrospinal fluids of patients. *Mem Inst Oswaldo Cruz* **2013**, 108 (1), 116-8.
77. Hidelarachi, M. D., et al., A case of eosinophilic meningitis following monitor lizard meat consumption, exacerbated by anthelmintics. *The Ceylon medical journal* **2005**, 50 (2), 84-6.
78. Sawanyawisuth, K., et al., A 1-week course of corticosteroids in the treatment of eosinophilic meningitis. *Am J Med* **2004**, 117 (10), 802-3.
79. Chotmongkol, V., et al., Treatment of eosinophilic meningitis with a combination of albendazole and corticosteroid. *Southeast Asian J Trop Med Public Health* **2004**, 35 (1), 172-4.
80. Moore, D. A., et al., Gnathostomiasis: an emerging imported disease. *Emerg Infect Dis* **2003**, 9 (6), 647-50.
81. Maleewong, W., et al., *Gnathostoma spinigerum*: growth and development of third-stage larvae *in vitro*. *J Parasitol* **1995**, 81 (5), 800-3.
82. Janwan, P., et al., Growth and development of *Gnathostoma spinigerum* (Nematoda: Gnathostomatidae) larvae in *Mesocyclops aspericornis* (Cyclopoida: Cyclopidae). *Parasit Vectors* **2011**, 4, 93.
83. Maleewong, W., et al., Growth and development of *Gnathostoma spinigerum* early third-stage larvae *in vitro*. *J Helminthol* **1997**, 71 (1), 69-71.
84. Funata, M., et al., Intraocular gnathostomiasis. *Retina* **1993**, 13 (3), 240-4.
85. Intapan, P. M., et al., Immunoblot diagnostic test for neurognathostomiasis. *Am J Trop Med Hyg* **2010**, 83 (4), 927-9.
86. Lv, S., et al., Human angiostrongyliasis outbreak in Dali, China. *PLoS Negl Trop Dis* **2009**, 3 (9), e520.
87. Sorvillo, F., et al., *Baylisascaris procyonis*: an emerging helminthic zoonosis. *Emerging infectious diseases* **2002**, 8 (4), 355-9.
88. Hochberg, N. S.; Hamer, D. H., Anisakidosis: Perils of the deep. *Clin Infect Dis* **2010**, 51 (7), 806-12.
89. Abollo, E., et al., *Anisakis* infestation in marine fish and cephalopods from Galician waters: an

- updated perspective. *Parasitol Res* **2001**, 87 (6), 492-9.
90. Lo Re, V.; Gluckman, S. J., Eosinophilic meningitis. *The American journal of medicine* **2003**, 114 (3), 217-23.
91. Takei, H.; Powell, S. Z., Intestinal anisakidosis (anisakiosis). *Annals of diagnostic pathology* **2007**, 11 (5), 350-2.
92. Morera, P., Life history and redescription of *Angiostrongylus costaricensis* Morera and Céspedes, 1971. *Am J Trop Med Hyg* **1973**, 22 (5), 613-21.
93. Nawa, Y., et al., Sushi delights and parasites: the risk of fishborne and foodborne parasitic zoonoses in Asia. *Clin Infect Dis* **2005**, 41 (9), 1297-303.
94. Lopez-Serrano, M. C., et al., Gastroallergic anisakiasis: findings in 22 patients. *Journal of gastroenterology and hepatology* **2000**, 15 (5), 503-6.
95. Lorenzo, S., et al., Usefulness of currently available methods for the diagnosis of *Anisakis simplex* allergy. *Allergy* **2000**, 55 (7), 627-33.
96. Audicana, M. T.; Kennedy, M. W., *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. *Clin Microbiol Rev* **2008**, 21 (2), 360-79, table of contents.



Arthur Looss, Ph.D. (1861–1923)

Looss completed the description of the life cycle of *Ancylostoma duodenale* by accidentally spilling a sample of hookworm larvae on his hand while trying to administer them to guinea pigs. He experienced itching at the site of the spill later that day. Later that month, Looss discovered eggs of *A. duodenale* in his stool, proving that the L3 larva is the infectious stage for humans and that it penetrates unbroken skin to initiate the infection. This seminal finding was to become the basis for controlling hookworm infection throughout the world at the community/public health level. This is a clear example of “luck favoring the prepared mind” (quotation from Louis Pasteur).

VI. The Cestodes

The phylum Platyhelminthes includes the class Cestoidea (tapeworms). All are parasitic, living in the gut tracts of various vertebrate hosts. Most tapeworm species have evolved a two-host or sometimes three-host strategy for completing their life cycle. Tapeworms are flat, segmented worms composed of a head (scolex), and a series of segments called proglottids. All proglottids of a given worm are collectively referred to as the strobila. The scolex is the point of attachment between the host and the parasite. It may be equipped with suckers, hooks, or grooves, which aid in attachment. The strobila contains nerves terminating in ganglia in the scolex. The neck region of the scolex is the site at which new proglottids originate. In a real sense, the adult worm behaves like a colony of independent organisms; the head remains attached to the host, while each proglottid matures, becomes gravid, and separates from the strobila, exiting the host with the feces.

Tapeworms do not have a gut tract. Each proglottid is surrounded by a specialized metabolically active tegument whose structure and function are directly related to nutrient acquisition. Evenly spaced microvilli cover the entire tegument surface, underneath which lie mitochondria, vesicles (perhaps involved in tegument replacement), and related structures. The tapeworm obtains some nutrients by transporting them across the tegument. Osmosis may also play a role in nutrient acquisition. Each proglottid absorbs various low-molecular-weight substrates, but its precise metabolic requirements have yet to be fully defined. The adult worm simultaneously expresses aerobic and anaerobic metabolic pathways. The tegumental surface is aerobic, while the internal portion of the proglottid is mainly anaerobic. Mitochon-

dria in each cellular compartment are consistent in the ultrastructural arrangement of cristae, adding supportive evidence from earlier physiological studies that revealed the dichotomy of these two metabolic strategies. High levels of ATPase in the tegument are related to active transport but may also help the worm resist digestion by the mammalian host. Inhibitors of tapeworm ATPase, such as niclosamide, cause disintegration of adult tapeworms by digestion in the presence of pancreatic secretions.

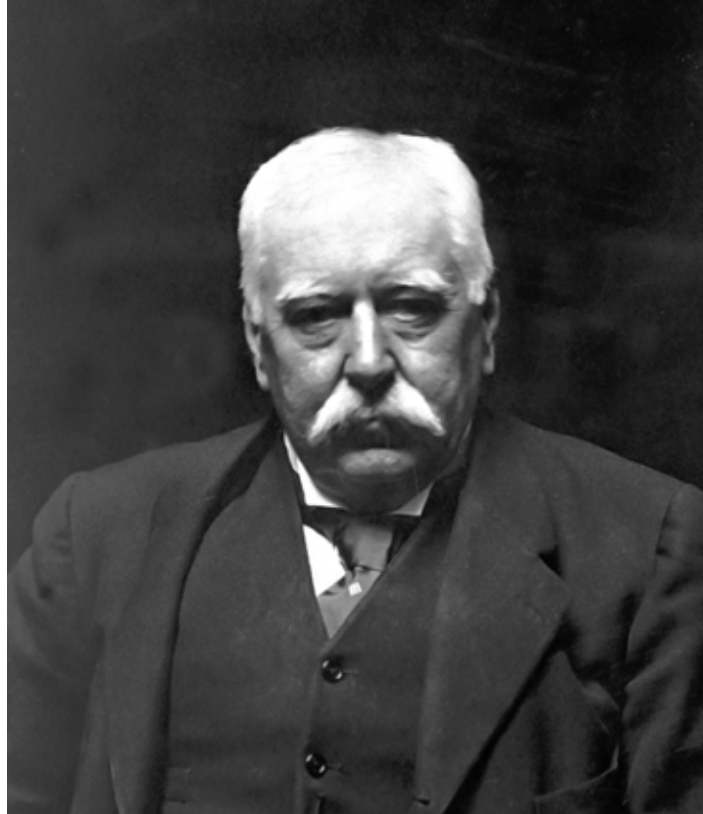
Each proglottid has two layers of muscle, longitudinal and transverse, enabling it to move. Two lateral branches of nerves innervate the worm, with perpendicular commissures branching out into the parenchyma of each segment. Segments are anatomically independent but are all connected by a common nervous system emanating from the central ganglia in the scolex. Osmoregulation and excretion of wastes occur in every proglottid via a lateral pair of excretory tubules.

Mature proglottids possess both male and female sex organs, but self-mating within a segment is unusual. Typically, sperm are transferred between adjacent mature proglottids. Gravid proglottids develop after mating and contain hundreds to thousands of embryonated eggs. The gravid proglottids then detach from the parent organism and exit via the host's anus. In some species, proglottids exit intact, while in others, segments disintegrate before leaving the host. Eggs are usually passed embryonated, containing a hexacanth larva called an oncosphere. Eggs may remain viable in the external environment for weeks to months after being deposited in soil. Hatching occurs typically within the small intestine of the intermediate host. The oncosphere then penetrates the gut tract and lodges within the tissues, developing into the metacestode (juvenile tapeworm). When

the definitive host ingests the juvenile stage, it transforms to the adult in the lumen of the small intestine.

Adult tapeworms do not cause significant pathology in the human intestine. Unlike adult nematodes or trematodes, adult cestodes do not adversely impact childhood

development. When humans serve as intermediate hosts, cestodes can cause significant morbidity. For example, neurocysticercosis caused by the juvenile stage of the pork tapeworm, *Taenia solium*, is a leading cause of neurologic disease; in many countries, it is the leading cause of epilepsy.



Patrick Manson, M.D. (1844–1922)

Manson was stationed on the island of Formosa (modern-day Taiwan) as a medical officer. During that time, he cared for a number of Chinese patients and became familiar with a wide variety of tropical infectious diseases, including lymphatic filariasis (LF). His investigations, as well as those of Thomas Bancroft, lead to the identification that culicine mosquitoes were the vectors of this nematode infection and he speculated that human malaria was spread in the same way. This was the basis for Ross's work in India, culminating in his discovery that bird malaria was transmitted by the same kind of mosquitoes. Manson also described two new species of helminth, infectious to humans, that today bear his name: *Schistosoma mansonii* (Trematoda), and the juvenile infection *Spirometra mansonoides* (Cestoda). Manson went on to become identified as the founder of modern tropical medicine.

28. *Taenia saginata*

(Goeze 1782)

Pronunciation: \tē-nē-ə\\sə-jə-nā-tə\

Introduction

Taenia saginata (\TEE-nee-ah\\SA-je-not-u\), along with *T. asiatica* and *T. solium*, belong to the order Cyclophyllidea, and are some of the largest parasites infecting humans, often achieving lengths approaching 8–10 m. Like all other adult tapeworms, they live in the lumen of the upper half of the small intestine. All three *Taenia* spp. are found exclusively in humans. They have no reservoir hosts. *Taenia saginata* occurs wherever cattle husbandry is prevalent and where human feces is not disposed of properly.^{1,2} It is commonly called the “beef tapeworm” because the juvenile stage of the parasite is found only in cattle, the intermediate host. Endemic foci include vast Sub-Saharan grasslands in Africa, particularly in Ethiopia,



Figure 28.1. A rare beef tenderloin.

due to the common dietary practice of ingesting raw beef (*kitfo*), large portions of Northern Mexico, Argentina, and, to a lesser extent, middle Europe. It is infrequently acquired in the United States, where most clinical cases are imported.

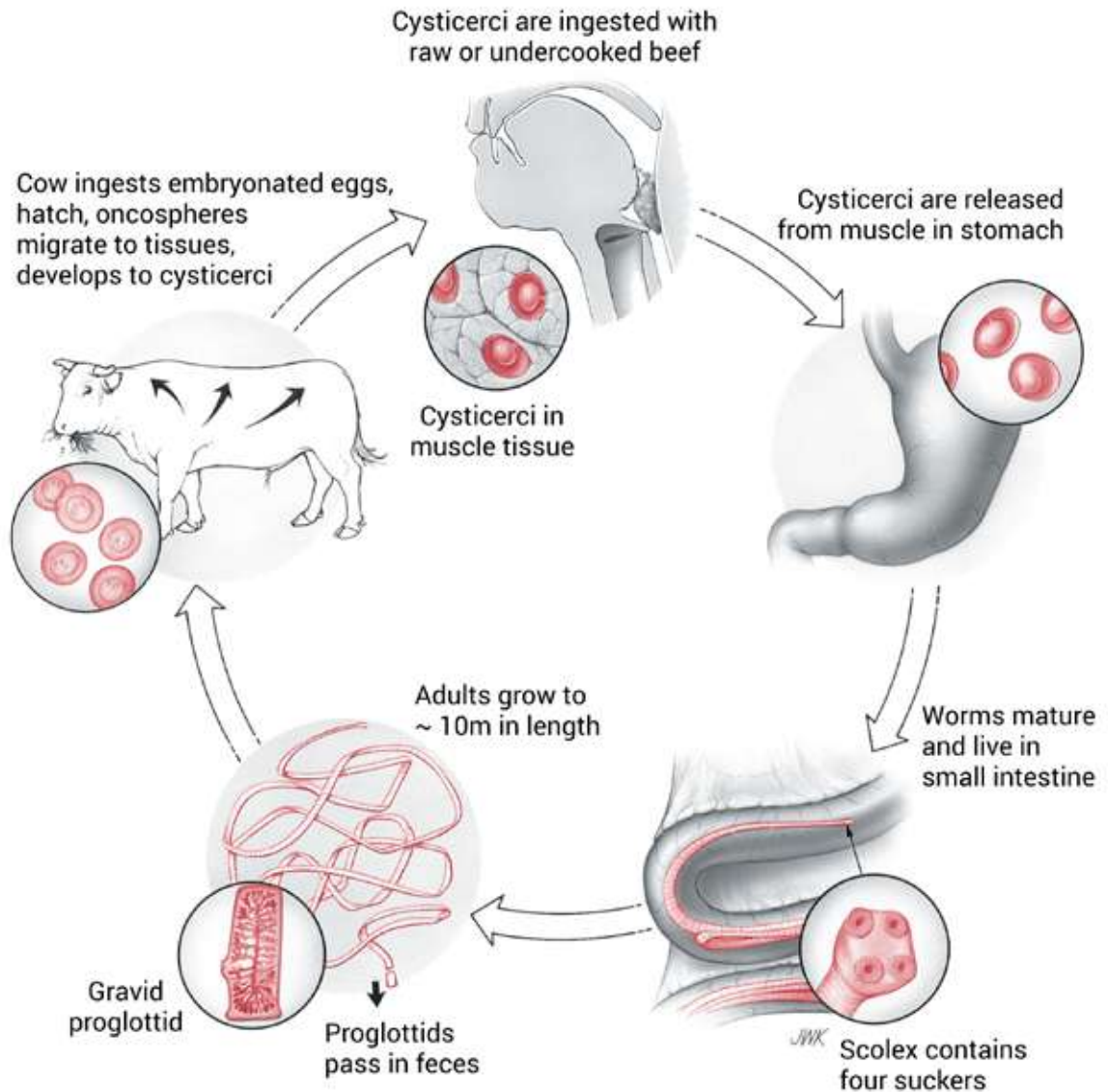
T. asiatica infects people in Taiwan, Korea, China, Vietnam, and Indonesia.^{3,5} The intermediate host of *T. asiatica* is the pig.^{5,6} The full clinical spectrum of disease caused by *T. asiatica* is still not fully appreciated.

Historical Information

The discovery of multiple species of tapeworms was initially intertwined. Many years after their initial description, this group of parasites was shown to consist of many species based on their detailed morphology and host specificity.⁹ Tyson described the tapeworms' head (scolex).⁹ In 1656, Félix Plater, a Swiss physician, wrote about the distinctions between *Taenia* spp. and *Diphyllobothrium latum* (then called *Lumbricus latus*).¹¹ In 1683, Edward Tyson, still working on tapeworms, described several species he recovered from dogs.¹⁰ Nicolas Andry de Bois-Regard is credited with the first report of adult *T. saginata* in humans in 1700. He didn't recognize that each proglottid was a separate unit and did not distinguish this worm from other similar tapeworms.¹²

In 1782, Johann Goeze accurately described the worm in a treatise on helminthology and suggested that *T. saginata* and *T. solium* adult worms were two different species.¹³ Two years later, Goeze speculated that an intermediate host was required to complete the life cycle when he noted that the scolices of tapeworms in humans resembled the juvenile stage present in the muscles of pigs.¹⁴ In 1850, Carl Von Siebold hypothesized that “bladder worms” (i.e., cysticerci) could either develop into adult tapeworms or per-

Taenia saginata



haps they were degenerated adults.¹⁵ In 1861, Friedrich Kuchenmeister fed meat from pigs containing cysticerci to condemned prisoners and then recovered the adult worms from these individuals' intestines after their execution.¹⁶ Two years later, Friedrich Leuckart reported on experiments showing that the proglottids of *T. saginata*, when fed to young calves, developed into cysticerci (juvenile tapeworms) in muscle tissue.¹⁷ John Oliver, in 1870, demonstrated that after human ingestion of "measly" beef, humans developed adult *T. saginata* infections and further suggested that thoroughly cooking infected meat would prevent infection with the adult tapeworm.¹⁸

Life Cycle

Infection begins when the cysticercus containing the juvenile stage is ingested in raw or undercooked beef (Fig. 28.1). The cyst enters the small intestine, and the cyst's wall is digested away, freeing the immature worm inside. The parasite then everts its scolex and attaches to the intestinal wall aided by four sucker disks (Fig. 28.2).

A mature adult tapeworm (Fig. 28.3) takes

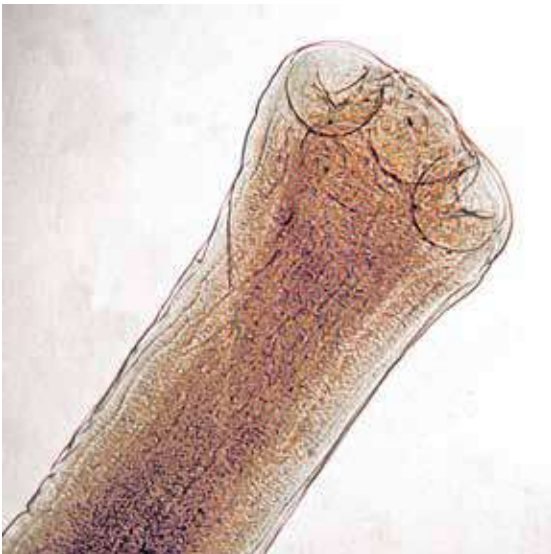


Figure 28.2. Scolex of *T. saginata*. Note 4 suckers.



Figure 28.3. Adult *T. saginata*. Note the position of the scolex (arrow). Courtesy of U. Martin.

about three months to grow to full length. The developing proglottids extend down the small intestine, sometimes reaching the ileum. All adult tapeworms feed by transporting nutrients (e.g., sugars, amino acids, nucleic acids) across their tegumental surface. Segments mature as they progress toward the terminal end of the worm. Terminal proglottids, gravid with hundreds of embryonated, infectious eggs, may detach from the colony and can actively migrate out the anus, where they may be deposited on the ground. Sometimes, whole ribbons of worms (20–30 segments) “escape” from an infected person.

To complete the life cycle, a cow must then ingest a gravid segment, usually along with grass or hay. Eggs lying inside the lumen of the uterine branches of the proglottid are freed from the tapeworm tissue by the cow's digestive enzymes, stimulating the hexacanth larva to hatch from the egg in the small intestine (Fig. 28.5). The hexacanth larva penetrates the intestinal wall, aided by its hooks and secretory peptidases.¹⁹ It enters the bloodstream and is passively carried throughout the body. Oncospheres can lodge in any of a variety of tissues, depending upon where the circulation takes them. Primarily, they infect striated skeletal muscle tissue. They encyst,

develop into the cysticercus (juvenile stage), and can live there for several years before calcifying.

Cattle can experience disease due to the space-filling lesions created by cysticerci, especially if the cysts develop in sensitive areas (i.e., neurological tissues).²⁰ Usually, they do not show signs of infection since cattle are routinely slaughtered within several years of birth, and cysticerci tend not to cause problems, even in neurological tissues (Chapter 32).

Cattle cannot become infected with adult parasites if they accidentally ingest cysticerci. Similarly, humans cannot harbor the juvenile stage since *T. saginata* eggs will only hatch in cows' stomachs. There is no risk of invasive disease in humans from the adult parasite. In contrast, cysticercosis due to the pork tapeworm, *T. solium*, can develop in humans, often resulting in severe disease (e.g., neurocysticercosis).

Most infected individuals harbor a single adult parasite, but there have been cases where numerous worms were recovered from a single infected individual. In these instances, the worms tend to be stunted due to crowding.²¹

Cellular and Molecular Pathogenesis

T. saginata occupies a large part of the lumen of the small intestine, but it is flexible and relatively fragile. Bowel obstruction does not occur. Adult worms are immunogenic, and specific serum antibodies are produced throughout the infection period, but local gut inflammatory responses are minimal.²² No serious pathological consequences result from even long-term infection with the adult worm.

In cattle, immunity to reinfection develops locally in the small intestine and is directed at newly arrived hexacanth larvae. Challenge infection can be prevented by vaccination of cows with recombinant egg antigens.²² In experimental infections, colostrum from immune cows protects sheep from invasion by hexacanth larvae of closely related species. Secretory IgA antibodies are thought to play a significant role in this protective response.²³

Clinical Disease

Most infections induce no symptoms, but some people may experience epigastric fullness. Rarely, postprandial nausea and vomiting occur, and individual cases of jejunal perforation and Meckel's diverticulitis have been reported.^{24,25} Infection is usually first detected by noting proglottids in stool. Frequently, proglottids migrate out of the infected person



Figure 28.4. Gravid proglottid of *Taenia saginata*.

overnight and can be discovered in bedding or clothing the following morning. A somewhat disturbing feature is that these proglottids move very similarly to inchworms.

Diagnosis (see Clinical Appendix)

One means of definitive diagnosis is by inspection of proglottids. Gravid proglottids can be fixed in 10% formaldehyde solution, and the uterus is injected with India ink with a 26-gauge needle (Fig. 28.4). *T. saginata* proglottids have 12 or more branches on either side of the uterus.²⁶ These uterine branches can be visualized using hematoxylin-eosin staining techniques.²⁶ Eggs of *T. saginata* are only occasionally found in stool since most proglottids usually pass out of the host intact. The species of *Taenia* cannot be determined by visual microscopy of the egg-based on morphology since all members of the family Taeniidae produce visually identical ova. Upon acid-fast staining, occasionally, the species can be distinguished, as fully mature eggs of *T. saginata* have an acid-fast shell.^{27,28}

Adult worms remain in the intestine. Only proglottid segments pass in the stool. It can be speciated on rare occasions when the scolex is obtained, such as after anti-parasitic therapy. *T. saginata*'s scolex has four lateral



Figure 28.5. *Taenia* spp. eggs. They cannot be differentiated from eggs of other members of the taeniid family.

suckers but no hooks. The scolex of *T. solium* also has four suckers, but it also has a double row of hooks.²⁶ Stool examination for eggs or proglottids is insensitive since segments and eggs are passed intermittently. Caution needs to be exercised by diagnostic parasitology technicians because the eggs of *T. solium* are infectious to humans. The sticky tape test is another diagnostically relevant test (see: diagnosis for *Enterobius vermicularis*). When proglottids migrate out of the anus, they express eggs that remain on the perineum.

T. saginata eggs can also be differentiated from *T. solium* and *T. asiatica* eggs by PCR.²⁹⁻³³ These molecular techniques have advanced, and loop-mediated isothermal amplification (LAMP) and multiplex PCR tests have become available.^{34, 35} An ELISA test has been developed to detect soluble *T. saginata* antigens in infected humans' stool samples (copro antigens).³⁶

Treatment (see Clinical Appendix)

Praziquantel (5–10 mg/kg) effectively treats *T. saginata* infection and often allows recovery of the intact scolex, confirming the cure.³⁷ Niclosamide is also effective in a single dose.^{38, 39} Niclosamide inhibits the parasite's ATPase, thus preventing it from interfering with host digestive enzymes. The consequence of treatment is the dissolution of the adult worm. A search for the scolex is futile. Quinacrine has also been reported to be effective for patients with niclosamide-resistant *T. saginata* infection, but it is not considered standard therapy.⁴⁰

Prevention and Control

Preventing tapeworm infection in the community is done by properly disposing of human feces. However, this has proven challenging to institute in some parts of the world

since untreated human feces is widely used as fertilizer. Proper education and adoption of composting approaches for human waste can destroy helminth eggs during the thermophilic phase and allow for using human waste as fertilizer while minimizing the risk of tapeworm infection from this source.⁴¹ Infection is entirely prevented by cooking beef thoroughly or freezing it solid before cooking. Unfortunately, people worldwide enjoy eating rare or raw beef, so infection control by thorough cooking is impractical in many countries. Meat inspection programs effec-

tively identify contaminated meat, but inspection is only done in some endemic areas.

A vaccine against the oncosphere of *T. saginata* for use in cattle has been developed and may prove useful in some endemic situations, but it is not yet available. The development of an ELISA test that detects antibodies in cattle specific for the cysticercus stage will allow for efficient evaluation of control programs, especially where good public health practices are coupled with meat inspection at the abattoir.⁴³

References

1. Ito, A., et al., The present situation of human taeniasis and cysticercosis in Asia. *Recent Pat Antiinfect Drug Discov* **2014**, 9 (3), 173-85.
2. Silva, C. V.; Costa-Cruz, J. M., A glance at *Taenia saginata* infection, diagnosis, vaccine, biological control and treatment. *Infect Disord Drug Targets* **2010**, 10 (5), 313-21.
3. Fan, P. C.; J., Annual economic loss caused by *Taenia saginata* asiatica Taeniasis in three endemic areas of east Asia. *Southeast Asian Med Public Health* **1997**, 28 Suppl 1, 217-21.
4. McManus, D. P.; J., Molecular genetic variation in *Echinococcus* and *Taenia*: an update. *Southeast Asian Med Public Health* **1997**, 28 Suppl 1, 110-6.
5. Ito, A., et al., Human Taeniasis and cysticercosis in Asia. *Lancet (London, England)* **2003**, 362 (9399), 1918-20.
6. Flisser, A., et al., Portrait of human tapeworms. *The Journal of parasitology* **2004**, 90 (4), 914-6.
7. Galan-Puchades, M. T.; Fuentes, M. V., Lights and shadows of the *Taenia asiatica* life cycle and pathogenicity. *Trop Parasitol* **2013**, 3 (2), 114-9.
8. Eom, K. S., What is Asian *Taenia*? *Parasitol Int* **2006**, 55 Suppl, S137-41.
9. Cox, F. E., History of human parasitology. *Clin Microbiol Rev* **2002**, 15 (4), 595-612.
10. Tyson, E., *Lumbricus latus*, or a discourse read before the Royal Society, of the joynted worm, wherein great many mistakes of former writers concerning it, are remarked; its natural history from more exact information is attempted; and the whole urged, as a difficulty against doctrine of univocal generation. *Philos Trans* **1683**, 13, 113-144.
11. Plater, F., *Praxeos Medicae Opus*. Basel **1656**.
12. Andry, N., *De la Generation des Vers dans le Corps de l'Homme* Paris. 1700.
13. Goeze, J. A. E.; Blankenburg, P. A., Versuch einer Naturgeschichte der Eingeweiderwumer Thierischer Korper. *Pape* **1782**.
14. Goeze, J. A. E., Neueste Entdeckung. Dass die Finnen im Schweinefleische keine Drusenkrankheit sondern wahre Blasenwurmer sind. Halle. **1784**.
15. Siebold, C. T. E., Von Uber den Generationswechsel der Cestoden nebst einer Revision der Gattung Tetrahyinchus. 1850.
16. Kuchenmeister, F., The *Cysticercus cellulosus* transformed within the organism of man into *Taenia solium*. *Lancet* **1861**, i, 39.
17. Leuckart, R., Die menschlichen Parasiten und die von ihnen her ruhenden Krankheiten. *Em Handund Lehrbuch fur Naturforscher und Arzte C E Wintersche Verlagshandlung Leipzig* **1863**.
18. Oliver, J. H., The importance of feeding the cattle and the thorough cooking of the meat, as the best preservatives against tapeworms. *Seventh Annual Report of the Sanitary Commissioner with*

- the Government of India Calcutta pp* **1871**, 82-83.
19. White, A. C., et al., *Taenia saginata* oncosphere excretory/secretory peptidases. **1997**, 82, 7-10.
 20. Oryan, A., et al., Clinico-pathological studies in cattle experimentally infected with *Taenia saginata* eggs. *Journal of the South African Veterinary Association* **1998**, 69 (4), 156-62.
 21. Despommier, D., People, Parasites, and Plowshares. 2014.
 22. Lightowlers, M. W., et al., *Taenia saginata*: vaccination against cysticercosis in cattle with recombinant oncosphere antigens. *Experimental parasitology* **1996**, 84 (3), 330-8.
 23. Gemmell, M. A., et al., Immunological responses of the mammalian host against tapeworm infections. IX. The transfer via colostrum of immunity to *Taenia hydatigena*. *Experimental parasitology* **1969**, 26 (1), 52-7.
 24. Jongwutiwes, S., et al., Jejunal perforation caused by morphologically abnormal *Taenia saginata* infection. *The Journal of infection* **2004**, 49 (4), 324-8.
 25. Chirdan, L. B., et al., Meckel's diverticulitis due to *Taenia saginata*: case report. *East African medical journal* **2001**, 78 (2), 107-8.
 26. Mayta, H., et al., Differentiating *Taenia solium* and *Taenia saginata* infections by simple hematoxylin-eosin staining and PCR-restriction enzyme analysis. *J Clin Microbiol* **2000**, 38 (1), 133-7.
 27. Ide, L., To differentiate *Taenia* eggs. *J Clin Microbiol* **2012**, 50 (8), 2836; author reply 2837.
 28. Jimenez, J. A., et al., Differentiating *Taenia* eggs found in human stools: does Ziehl-Neelsen staining help? *Trop Med Int Health* **2010**, 15 (9), 1077-81.
 29. Gonzalez, L. M., et al., Differential diagnosis of *Taenia saginata* and *Taenia solium* infections: from DNA probes to polymerase chain reaction. *Trans Trop Med Hyg* 96 Suppl **2002**, 1, S243-50.
 30. Gonzalez, L. M., et al., Differential diagnosis of *Taenia saginata* and *Taenia solium* infection by PCR. *Microbiol* **2000**, 38, 737-44.
 31. Rodriguez-Hidalgo, R., et al., Comparison of conventional techniques to differentiate between *Taenia solium* and *Taenia saginata* and an improved polymerase chain reaction-restriction fragment length polymorphism assay using a mitochondrial 12S rDNA fragment. *The Journal of parasitology* **2002**, 88 (5), 1007-11.
 32. Nunes, C. M., et al., *Taenia saginata*: polymerase chain reaction for taeniasis diagnosis in human fecal samples. *Experimental parasitology* **2003**, 104 (1-2), 67-9.
 33. Gonzalez, L. M.; Montero, E., Differential diagnosis of *Taenia saginata* and *Taenia saginata asiatica* Taeniasis through PCR. *Diagn Microbiol Infect Dis* **2004**, 49, 183-8.
 34. Sako, Y., et al., Loop-mediated isothermal amplification method for a differential identification of human *Taenia* tapeworms. *Methods Mol Biol* **2013**, 1039, 109-20.
 35. Nkouawa, A., et al., A loop-mediated isothermal amplification method for a differential identification of *Taenia* tapeworms from human: application to a field survey. *Parasitol Int* **2012**, 61 (4), 723-5.
 36. Deplazes, P., et al., An enzyme-linked immunosorbent assay for diagnostic detection of *Taenia saginata* copro-antigens in humans. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1991**, 85 (3), 391-6.
 37. Pawlowski, Z. S., Efficacy of low doses of praziquantel in taeniasis. *Acta Trop Basel* **1991**, 48, 83-88.
 38. Keeling, J. E. D., The chemotherapy of cestode infections. *Adv Chemother* **1978**, 3, 109-152.
 39. Vermund, S. H., et al., Taeniasis unresponsive to a single dose of niclosamide: case report of persistent infection with *Taenia saginata* and a review of therapy. *Reviews of infectious diseases* **1986**, 8 (3), 423-6.
 40. Koul, P. A., et al., Mepacrine therapy in niclosamide resistant taeniasis. *The Journal of the Association of Physicians of India* **2000**, 48 (4), 402-3.
 41. Kone, D., et al., Helminth eggs inactivation efficiency by faecal sludge dewatering and co-composting in tropical climates. *Water Res* **2007**, 41 (19), 4397-402.
 42. Lightowlers, M. W., et al., Vaccination against cestode parasites: anti-helminth vaccines that work and why. *Veterinary parasitology* **2003**, 115 (2), 83-123.
 43. Onyango-Abuje, J. A., et al., Diagnosis of *Taenia saginata* cysticercosis in Kenyan cattle by antibody and antigen ELISA. *Veterinary parasitology* **1996**, 61 (3-4), 221-30.



Francisca Mutapi, Ph.D. (current)

Mutapi was born and grew up in Zimbabwe and then went on to study at the University of Oxford as a Beit Trust Scholar. After completing postdoctoral training at the Institute of Tropical Medicine in Antwerp, she went on to become a leader in the emerging field of global health with a focus on tropical diseases. Dr. Mutapi is credited with prioritizing schistosomiasis as a public health concern for the World Health Organization and has championed a multidisciplinary approach to global health that incorporates economic, cultural, technological and evidence-based challenges to global health problems. Dr. Mutapi is also credited with advancing our understanding of the immune responses to helminths.

29. *Taenia solium* (Linnaeus 1758)

Pronunciation: \tē-nē-ə\\sōl-ī-əm\

Introduction

Taenia solium (\TEE-nee-ah\\soh-lee-um\) belongs to the order Cyclophyllidea, and its occurrence is associated with the raising of pigs. It is commonly known as the “pork tapeworm”, although this worm only reaches sexual maturity as an adult in humans.^{1,2} In order to transmit *T. solium*, human feces, contaminated with the gravid segments of the worm, must be a regular contaminant of the pigs’ environment and coincident with the habit of eating raw or undercooked pork products. These conditions exist in many parts of the world.

T. solium is a large tapeworm, often achieving lengths of ~10 meters. It lives in the lumen, attached to the wall of the small intestine. There are no reservoir hosts for this parasite.³ Unlike the egg of *Taenia saginata*, the

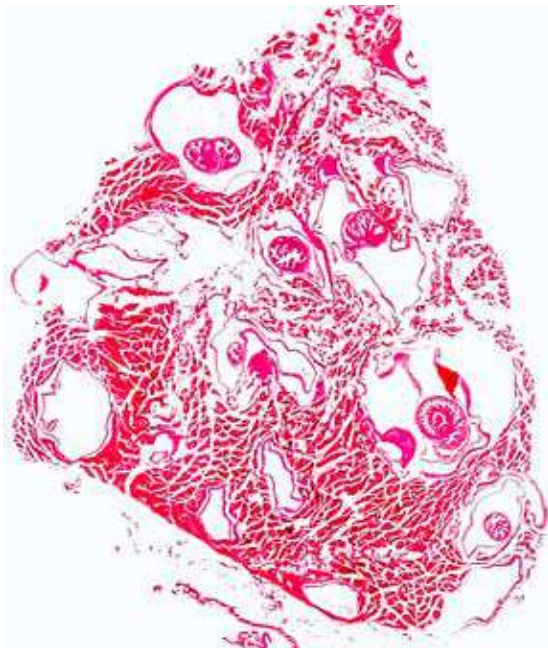


Figure 29.1. A section of infected pig muscle. Note numerous cysticerci.

egg of *T. solium* can infect the human host, resulting in a condition called cysticercosis. The juvenile stage can cause serious clinical disease and even be fatal, particularly if the larval tapeworm invades the CNS.^{4,5} Neurocysticercosis ranks among the most common causes of acquired epilepsy worldwide.⁶

T. solium is endemic in most of South America (particularly in the Andean region and Brazil), Central America, Mexico, China, the Indian subcontinent and Southeast Asia, Sub-Saharan Africa (particularly, Burundi, Tanzania, Democratic Republic of Congo, and South Africa), and Eastern Europe.^{7,8} It is an issue worldwide due to the high number of imported cases seen outside the endemic areas.^{4,5,9} In some of these endemic regions, up to 6% of the population may harbor *T. solium* adult tapeworms.⁶ The highest prevalence of *T. solium* infection in the United States occurs among Hispanic populations in Southern California, New Mexico, and Texas. This is because of the large number of immigrants from some endemic areas mentioned above.^{6,10,11} Among adult migrant workers in California, for instance, the sero-prevalence of *T. solium* is approximately 1–2%.¹² In endemic regions, *T. solium* occurs more commonly in children and adolescents.⁶ In addition to imported infections, it is believed that autochthonous transmission of *T. solium* infection also occurs in the United States.¹⁴ One estimate suggests that tens of thousands of cases occur in the United States, but there is a need for better surveillance and disease detection to obtain a more precise estimate.¹⁵

In 2003, the Fifty-sixth World Health Assembly highlighted the public health threat caused by *T. solium* and urged its member states to increase measures to control this infection.¹⁰ In 2012, the World Health Organization, the Food and Agriculture Organization, and the United Kingdom’s Department for International Development included *T. solium*

among the 17 neglected zoonotic diseases that they felt could be targeted for effective control.^{16, 17}

Historical Information

In 1688, Philip Hartmann described cysticercosis in pigs.¹⁸ In 1861, Friedrich Kuchenmeister, fed meat from pigs containing tapeworm cysticerci to condemned prisoners and then recovered the adult worms from these individuals' intestines 120 hours after death at autopsy.^{19, 20}

Life Cycle

The life cycle phase that occurs in humans depends on whether a human ingests *T. solium* eggs or encapsulated cysticerci in undercooked meat. Intestinal infection in the human host begins following the ingestion of raw or undercooked pork that harbors the worm's encapsulated cysticercus (juvenile stage) (Figs. 29.1, 29.2). The capsule is digested in the stomach, freeing the juvenile parasite. Upon entering the small intestine, the worm everts its scolex and attaches to the intestinal wall aided by four suckers and a crown of hooklets. In an experimental infection of hamsters with the juvenile stage of *T. solium*, the attachment site in the gut tract was studied.²¹ The hooklets penetrated the intestinal wall, while the four suckers all attached to cells of the surrounding villi. Host cell damage was observed in and around each sucker disk.

The intestinal phase of the life cycle of *T. solium* is very similar to that of *T. saginata*. The juvenile parasite develops to the adult (Fig. 29.3) in the small intestine over three months, after which gravid (egg-laden) proglottids begin passing from the host. Adult *T. solium* scolices closely resemble the adult worm of *T. saginata*, except that *T. solium* scolex possesses two rows of hooklets, in



Figure 29.2. Isolated whole cysticercus of *Taenia solium*, measuring 2–5 mm in diameter.

addition to four sucker disks (Fig. 29.4). Usually, adult *T. solium* do not live in the human gastrointestinal (GI) tract for more than 5 years.⁶ Humans are the only definitive host, and the adult of *T. solium* will not develop in the pig if it accidentally ingests cysticerci from contaminated pork scraps.

Unlike infection with *T. saginata*, the tissue phase of the life cycle can occur in humans and pigs following ingestion of embryonated *T. solium* eggs (Figs. 29.5, 29.6). When the egg is ingested, the larva (oncosphere) inside survives the stomach's gastric acid and enters the small intestine. The egg hatches and the larva penetrates the intestinal wall and enters the bloodstream. Eventually, the oncosphere penetrates into one of many tissues (e.g., striated muscles, heart, brain, eye) and encysts there. The oncosphere rapidly differentiates into a cysticercus (Fig. 29.7), grows, develops, and creates a space-filling lesion within 2–3 months, typically measuring approximately 10 mm³. Cysticerci achieve maximum



Figure 29.3. Whole adult *T. solium*. Arrow indicates scolex.

size about three weeks after entering a given tissue, and then cease growing. In some cases, it is thought that humans auto-infect with the contaminated eggs of their own tapeworm. The frequency of *T. solium* auto-infection is not known, although it has been observed that up to 15% of patients with cysticercosis also harbor an adult *T. solium* tapeworm.⁶

Cellular and Molecular Pathogenesis

The adult parasite living in the intestinal lumen does not usually cause a significant host inflammatory response but elicits the formation of humoral antibodies.²² Infected pigs harboring the juvenile stage are less likely to become re-infected when they ingest more eggs, most likely because they are protected by immune responses directed against the oncosphere.²³ A similar situation exists between the cysticercus of *T. sagi-*

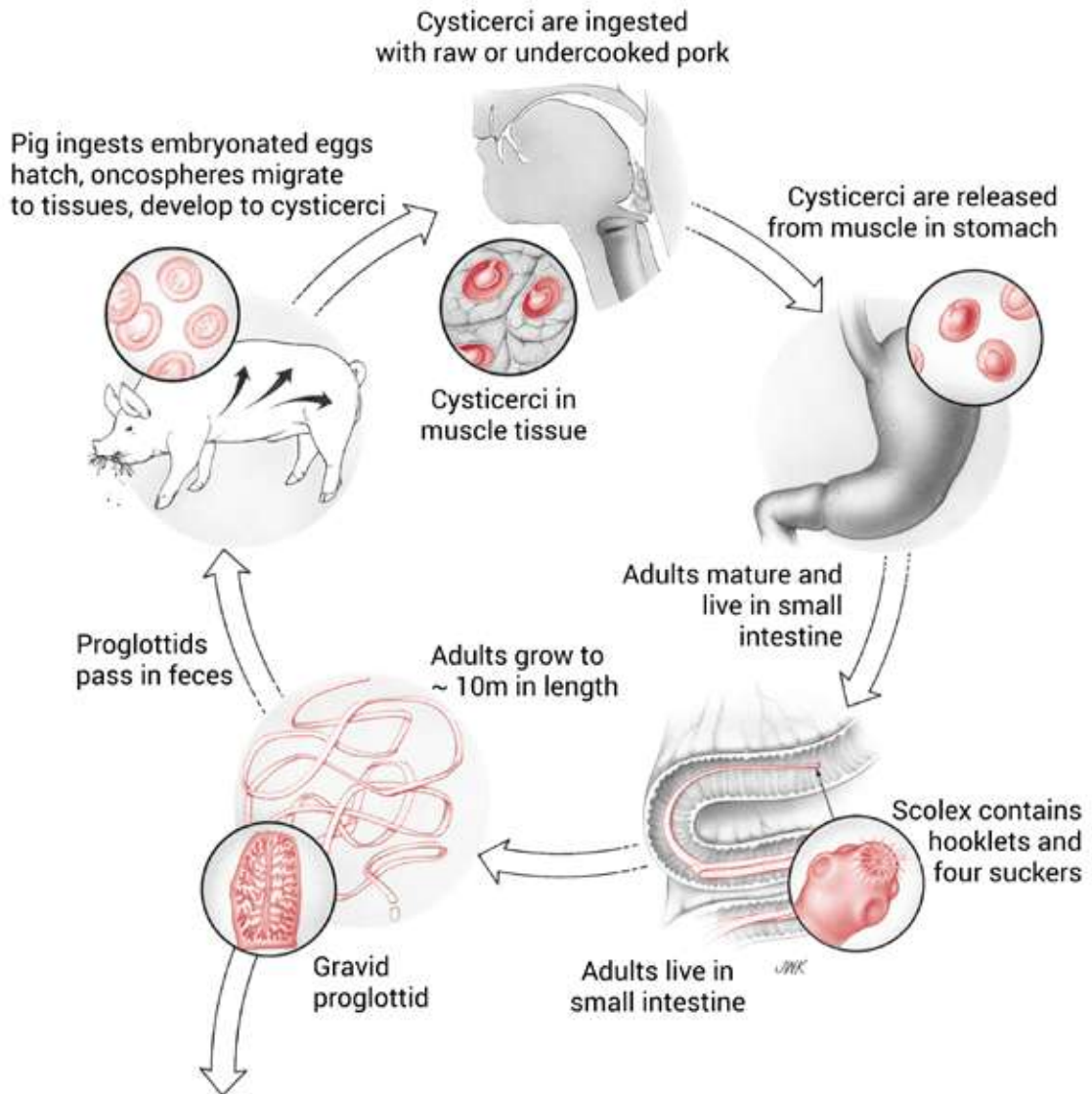
nata and cattle. The cDNAs encoding two *T. solium* glucose transporter molecules have been expressed in bacteria, and their fusion proteins have been identified and characterized.²⁴ One is on the tegumental surface of the adult worm (TGTP1), and the other is on the surface of the hexacanth larva (TGTP2). Both molecules can potentially be used as targets of vaccines aimed at preventing each stage from accessing host glucose. Vaccines using recombinant *T. solium* oncosphere larval antigens TSOL18, TSOL45-1A and TSOL16 produced in *E. coli* have been studied with demonstrated efficacy in preventing infection in pigs, both under controlled experimental conditions and in field trials.^{25,26}

Much of the pathological consequences in the tissue phase are those of a space-occupying lesion and the inflammation that results from death of the parasites.²⁷ Oncospheres

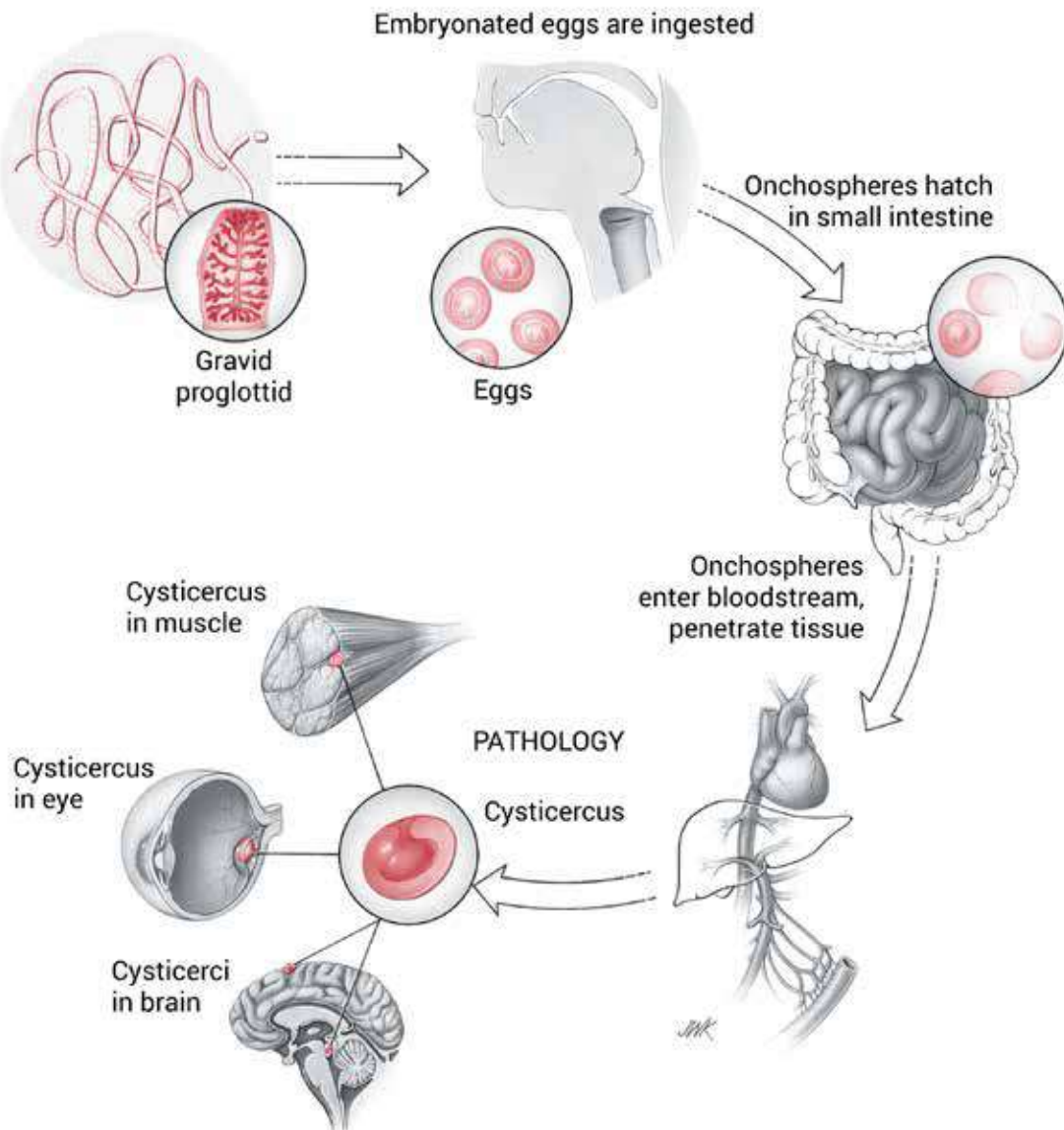


Figure 29.4. Scolex of *Taenia solium*. Note four suckers and hooks. Photo E. Gravé.

Taenia solium



Cysticercosis (*Taenia solium*)



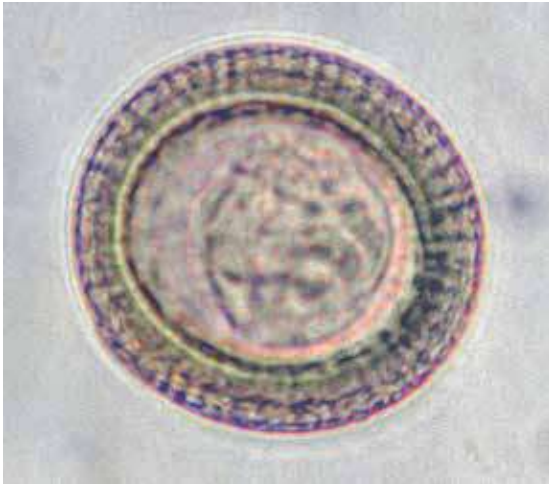


Figure 29.5. *Taenia* spp. egg. The species of tapeworm cannot be determined based on its morphology. Eggs measure 30–40 μm in diameter.

that lodge in the CNS (e.g., eye, and brain; Figs. 29.8, 29.9) develop with little immune response until the parasites die, triggering an intense immune response accompanied by edema, and in many cases, seizures.²⁸

Oncospheres, other than those in the CNS, are presumably more susceptible to immune attack. Their rapid development to the cysticercus stage may account for the high survival rate, at least during a primary infection. Cysticercus development is coincident with the modulation of host immune responses, favoring the expression of parasite immune evasion mechanisms that interfere with Th2-type protective immune responses.²⁸ Immunomodulatory substances have been identified and partially characterized; for example, Taeniastatin, a protease inhibitor, and paramyosin, which inhibit different aspects of the complement cascade.^{29–31}

Ultimately, cysticerci die after several years and become calcified. Dying parasites release their suppressive hold on protective host immune responses, and antigens are released. When cellular reactions are present, they are partly due to interleukin signaling from T-cells. Specific IgM antibodies and natural killer cells are also present in these

infiltrates.³² An acute local inflammatory reaction follows, consisting of lymphocytes, plasma cells, and eosinophils, which may be clinically significant in neurocysticercosis.²⁸ This host inflammatory response around degenerating cysticerci, when it occurs in the brain, may be responsible for eliciting seizures and intracranial hypertension. Neuroimaging studies (CT with contrast or MRI) reveal the inflammation encircling the lesion, giving rise to the appearance of “ring enhancement.” In addition, cysticerci occurring in anatomically sensitive sites in the CNS can cause mass effect, or block the circulation of cerebrospinal fluid (CSF) leading to hydrocephalus.⁶

Strong, protective immunity to invasion of tissues by oncospheres can be induced in a variety of animals, and these findings offer hope for developing a vaccine for use in pigs and possibly humans.³³

Clinical Disease

There are two distinct patterns of infection with *T. solium*: intestinal infection with the adult parasite following ingestion of the juvenile stage in raw or undercooked pork, and systemic infection following ingestion of embryonated eggs from a human with an intestinal infection. Intestinal infection with



Figure 29.6. Commercial feedlot. This environment is frequently contaminated with human feces in many regions of the world where pigs are raised. Courtesy P. M. Schantz.



Figure 29.7. Cysticercus of *Taenia solium*.

adult *T. solium* causes few symptoms other than epigastric fullness in some individuals. Infected individuals are asymptomatic and are unaware of their infection until they discover the proglottids in stool, perianal skin, clothing, or bed sheets. Some people report abdominal pain, distension, diarrhea, and nausea, but no controlled studies demonstrate their link with *T. solium* in the gut. These symptoms may be due to other causes.⁶ Two patients with *T. solium* infection were reported to develop ascites, chronic diarrhea, and malabsorption, which resolved following antihelminthic therapy. But their connection to infection with *T. solium* remains unresolved.³⁴

Invasive infection has two major clinical presentations: extraneural cysticercosis (subcutaneous and intramuscular) and neurocysticercosis (parenchymal and extraparenchymal).⁶ These two conditions are not mutually exclusive, as most patients with neurocysticercosis also have evidence of infection in other parts of the body.⁶

Extraneural cysticercosis is usually asymptomatic, but infected individuals may notice painless subcutaneous nodules in the arms, chest, or small, discrete swellings of particu-

lar muscles (Fig. 29.10), in which cysticerci have lodged.³⁵ After several months, nodules become swollen and tender, presumably because of the host's inflammatory response to the cysticerci. Subsequently, the nodules disappear. This clinical presentation may have geographic variation because subcutaneous cysticercosis is common in Asia and Africa, but rare in Latin America.⁶ Ultimately, these lesions will calcify after the death of the parasite. The calcifications can persist for years. Some people with extraneural cysticercosis can also harbor cardiac cysticerci, usually asymptomatic.⁶

Neurocysticercosis is the most severe manifestation of systemic infection with *T. solium*. The signs and symptoms vary greatly with the number and distribution of cysts (Figs. 29.8 – 29.13).³⁵ It often presents as a space-occupying lesion, and, in many respects, mimics a tumor.²⁷ Seizures, hydrocephalus, and focal neurologic abnormalities may be the presenting signs.³⁶ It has been estimated that seizures occur in 50–80% of patients with brain parenchymal cysts, resulting from host inflammation around dead and degenerating parasites.^{6,37} In endemic regions, teenagers and young adults exhibit the highest rates



Figure 29.8 Cysticercus of *Taenia solium* floating free in the anterior chamber of the eye.



Figure 29.9. Cysticercus of *Taenia solium* in brain.

of *T. solium* seizures.⁶ These patients, especially females, can also develop an accompanying encephalitis.⁶ Children immigrating to the United States from endemic areas may present with a solitary mass or multiple lesions and seizures.³⁸ Usually, with those suffering seizures, the cysticercus has begun to degenerate and, in some cases, started to calcify (Fig. 29.13).³⁹

People still residing in endemic areas frequently have a more complex disease and a greater likelihood of presenting with multiple cysts, including cysts that locate in the subarachnoid space or the ventricles.⁶ These patients present with elevated intracranial pressure, arachnoiditis, meningitis, encephalitis, or hydrocephalus.⁴⁰ They are also more likely to experience stroke.

There appear to be geographic differences in the presentation of clinical neurocysticercosis. In Asia, patients most commonly present with a single enhancing brain lesion, whereas in Latin America it is common to find mul-

tiples cysts without evidence of inflammation.⁶

Extraparenchymal neurocysticercosis with disease in the ventricles, subarachnoid space, and the eye is less common. These infections generally increase the risk of morbidity and mortality, largely through the elevation of intracranial pressure. Ophthalmic cysticercosis occurs in approximately 1–3% of all infections and most typically manifests as intraocular cysts floating freely in the vitreous humor or the subretinal space. Patients complain of seeing shadows. Subsequent development of uveitis, retinitis, or choroidal atrophy can lead to visual loss.⁶

Diagnosis (see Clinical Appendix)

Diagnosis of the adult phase can be made by identifying the *Taenia* eggs by micros-



Figure 29.10. Radiogram of lower leg with numerous cysticerci of *Taenia solium*.

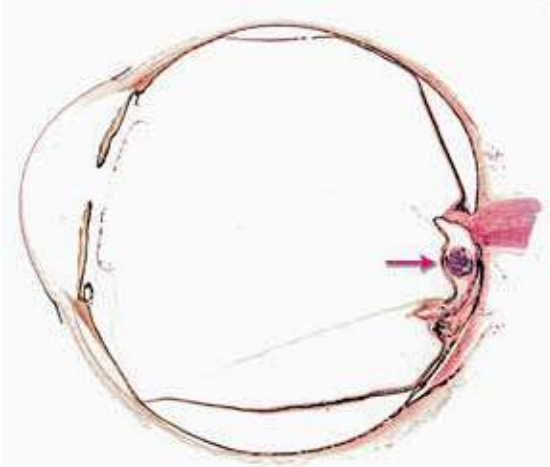


Figure 29.11. Cross section of whole eye with cysticercus of *T. solium* (arrow) in retina at the level of the optic nerve.

copy, by analysis of the gravid proglottids, or by recovering and identifying the scolex in feces. Overall, the sensitivity of stool microscopy is poor. *T. solium* eggs are morphologically indistinguishable from *T. saginata* eggs. Upon acid-fast staining, the two species can occasionally be distinguished, as fully mature eggs of *T. saginata* have an acid-fast shell, and eggs of *T. solium* do not stain.^{41, 42} A DNA dot blot test can differentiate *T. solium* from *T. saginata*.⁴³ NAAT and fecal antigen tests are available for diagnosing and differentiating *T. solium* eggs from *T. saginata*.⁴⁴⁻⁴⁶ Perianal scraping with clear adhesive tape to capture eggs has been used to improve the sensitivity of microscopy. But this technique is not as effective for *T. solium* infection as compared to *T. saginata*.

Gravid proglottid segments can be fixed in 10% formaldehyde solution and injected with India ink to fill the uterus. Proglottids of *T. solium* have less than 12 uterine branches per side (Fig. 29.14), compared to those of *T. saginata*, which have 12 or more (Fig. 28.4). Proglottid segments can also be prepared and viewed using standard hematoxylin and eosin staining. Extreme caution must be exercised when handling unfixed proglottids of *T.*

solium, since the embryonated eggs are infectious.

Morphological features of the scolex of *T. solium* and *T. saginata* can be used to diagnose both species. Polyethylene glycol salt purges to improve bowel emptying significantly improves the likelihood of scolex recovery but is not often used.⁴⁷

ELISA performed on fecal extracts for antigen detection is sensitive (95%) and specific (99%).⁴⁸ Patients with adult tapeworms also produce antibodies detectable by immunoblot assay.⁴⁹ Serological assay is particularly useful for epidemiological studies.

Diagnosis of cysticercosis is usually based on imaging tests, and criteria have been established.^{6, 40, 50} Neuroimaging by CT or MRI is critically important in diagnosing



Figure 29.12. Alien invader: enlarged view of cysticercus from Figure 29.11.



Figure 29.13. Radiogram of brain with calcified lesion (arrow) due to infection with the metacestode of *Taenia solium*.

neurocysticercosis. Ideally, both modalities are employed, combining the advantage of higher sensitivity of MRI with CT detection of calcium. Because it is often not practical to biopsy cysticerci, consulting a neuroradiologist is extremely helpful. On CT, the cysts appear as hypodense images containing a small hyperdense nodule representing the parasite scolex. At times, calcium can be observed. Inflammation surrounding dead and dying parasites will provide so-called ring enhancement in the presence of contrast media. In the natural history of neurocysticercosis, the dead and dying cysts calcify. MRI is more expensive but is becoming more available in developing countries, as it often provides a clearer image of the cysticercus and has greater sensitivity for multiple lesions.⁶

Serologic testing provides important confirmatory data for patients with suspicious lesions on CT or MRI. Serological testing assays have improved, with a sensitivity of 98% and a specificity approaching 100%.^{6, 51-53} For patients with a solitary lesion, the sensitivity is much lower, presumably because of the small amount of parasite antigen available to the host immune system. In an acute care setting, the ELISA requires considerable interpretation because antibody titers can remain high long after the death of the parasites.⁶ Clinical practice guidelines that outline specific diagnostic criteria were updated in 2018.⁵⁴

Treatment (see Clinical Appendix)

Before initiation of treatment for any *Taenia* infection, it is important to look for the presence of neurocysticercosis. Praziquantel is the drug of choice for adult *T. solium*. Its use must be tempered by the possibility that this treatment will destroy occult cysticerci in the brain and trigger CNS manifestations.⁶ If neurocysticercosis has either been effectively ruled out or treated, then treatment for



Figure 29.14. Gravid proglottid of *Taenia solium*. Uterus contains less than 12 lateral branches on each side.

the intestinal worms can proceed. Praziquantel is usually available in developing countries, particularly because of its use for global schistosomiasis control, and its proven success in Mexico in reducing the prevalence where taeniasis is endemic.¹⁰ No purgatives should be used because this increases the risk of regurgitating eggs into the stomach, initiating infection and leading to cysticercosis.⁵⁵ ⁵⁶ Niclosamide is an alternative for adult *T. solium* when praziquantel is unavailable (2 g orally in a single dose).^{6,57}

Treatment of neurocysticercosis is based on the number of lesions, the stage of these lesions, and their location. Lesions can be single or multiple. The lesions may be viable (vesicular stage), degenerate (the colloidal stage), collapsed (granular nodular stage), or nonviable (calcified).⁵⁸ The lesions can be either intraparenchymal or extraparenchymal (vesicular). Seizures most often occur in neurocysticercosis when the host inflammatory response is activated as parasites begin to die. In this situation, initiation of therapy can trigger edema with resultant seizure activity.⁶ Increasingly, the complexities of the medical and surgical management of neurocysticercosis require a team approach and the addition of anti-inflammatory agents such as corticosteroids. Typically, this might include an infectious disease specialist, a neurologist, a neuroradiologist, an ophthalmologist, and a neurosurgeon.

Albendazole is used to treat neurocysticercosis when only 1-2 parenchymal cysts are seen on MRI while albendazole combined with praziquantel is preferred when there are larger numbers of parenchymal cysts although drug interactions between praziquantel with corticosteroids and seizure medications need to be managed.^{59, 60, 61} The results of two double-blind, placebo-controlled trials in patients with a solitary enhancing lesion, and a third double-blind, placebo-controlled

trial comparing the evolution of brain parenchymal lesions on CT after three months of treatment were summarized by Garcia.⁶ Only one of these trials showed benefit from albendazole in terms of resolution of lesions and seizures. The current recommendation is that therapeutic decisions on using albendazole (15 mg/kg daily for 10-14 days) with or without praziquantel (50 mg/kg for 10 days) need to be tailored to the individual patient, based on the number, location, and viability of the cysticerci.^{6, 61} All patients selected for treatment with antihelminthic drugs should undergo a prior ophthalmologic exam to rule out intraocular cysts and be counseled about the risk of treatment-induced seizures.

The inflammatory response in the brain is responsible for seizures, encephalitis, and elevated intracranial pressure. Both albendazole and praziquantel kill cysticerci and exacerbate brain inflammation and edema thus patients being treated for neurocysticercosis also require steroids, usually dexamethasone, to reduce swelling. Dexamethasone affects the blood levels of both praziquantel and albendazole, so drug doses of both may need to be adjusted.⁶² Additional medical management with anticonvulsants is also critical to both the acute and long-term management of *T. solium*-induced seizures.

Neurosurgical management with the placement of ventricular shunts is often required for complicated neurocysticercosis involving cerebrospinal obstruction and hydrocephalus. Surgical management may also be required for spinal and eye lesions.

Prevention and Control

Taenia solium is a significant public health problem, even outside the endemic areas, due to the association of cysticercosis with adult infections.⁶³ For example, an outbreak of cysticercosis was reported among an orthodox

Jewish community in New York City, resulting from ingesting *T. solium* eggs passed from domestic employees who were recent emigrants from Latin America.⁶⁴ It has been suggested that recent emigrants from countries in which *T. solium* infection is endemic should be screened for tapeworm infection before they are employed as housekeepers or food handlers.⁶⁴

In many impoverished regions in developing countries, pigs are more affordable than cows since pigs are omnivores. That is why owners allow them to roam free to eat garbage and, perhaps, human feces.⁶ The pig infection is preventable by protecting their feed from contamination with human feces. Individual infection is prevented by thoroughly cooking pork or by freezing it at -10 °C for a minimum of 5 days. Cysticerci can survive in meat refrigerated at 4 °C for up to 30 days.⁶⁵

In 2003, the World Health Assembly identified several measures to control *T. solium* infection, including: 1. identification and treatment of individuals who carry the adult tapeworm, 2. universal or selected treatment with praziquantel to reduce the prevalence in areas where *T. solium* infection is endemic, 3. veterinary sanitary measures, such as enforced meat inspection and control, 4. improvement of pig husbandry, and treatment of infected animals with single-dose therapy, and 5. case management, reporting, and surveillance of people with cysticercosis. Community-based sanitation and pig management interventions prove highly effective in disease control.⁶⁶ Ring-screening strategies have been proposed to control *T. solium* infection.⁶⁷

Anthelmintic chemotherapy programs must be linked with other health programs using a wider integrated approach.¹⁰ In 2012, *T. solium* was added by the World Health Organization to their list of neglected zoonotic diseases that they felt could be targeted for effective control.^{16, 17} The 2020 WHO NTD Roadmap articulates an integrated approach to cysticercosis disease mitigation and elimination with community health education on disposal of human feces and veterinary public health to interrupt the cycle of human and pig infection.⁶⁸ Inoculation of pigs with recombinant antigens cloned from parasite oncosphere mRNA appears to be an effective vaccine, and could be used to reduce the incidence of adult tapeworm in a few countries, such as Mexico and Uganda,⁶⁹ where public health programs can be integrated with prevention on the farm.^{33, 70-72} In one experimental study, treating all pigs with oxfendazole eliminated 100% of viable cysticerci.⁷³ Serological testing in the abattoir is now also possible.⁷⁴ Any of these three approaches, or in combination, could significantly reduce transmission of *T. solium* to humans if applied rigorously to commercial pig farms in endemic areas.

Some black bears in California have acquired cysticercosis, apparently as the result of feeding at human fecally contaminated garbage dumps near campgrounds.⁷⁵ Hunting bears is a popular sport in the United States, and distributing meat from kills to neighbors is common among hunters. Hunter organizations should issue warnings, advising hunters that any meat obtained from carnivores or omnivores should be well-cooked before eating.

References

1. Bush, A. O., et al., Parasitology meets ecology on its own terms: Margolis et al. revisited. *J Parasitol* **1997**, *83* (4), 575-83.
2. Pojmanska, T., [A review of ecologic terms used in current parasitology]. *Wiad Parazytol* **1993**, *39* (3), 285-97.
3. Pawlowski, Z., et al., Control of *Taenia solium* taeniasis/cysticercosis: from research towards implementation. *Int J Parasitol* **2005**, *35* (11-12), 1221-32.
4. Coyle, C. M., Neurocysticercosis: an update. *Curr Infect Dis Rep* **2014**, *16* (11), 437.
5. Coyle, C. M., et al., Neurocysticercosis: neglected but not forgotten. *PLoS Negl Trop Dis* **2012**, *6* (5), e1500.
6. Garcia, H. H., et al., The Cysticercosis Working Group in Peru. *Lancet* **2003**, *361*, 547-56.
7. Nsengiyumva, G., et al., Cysticercosis as a major risk factor for epilepsy in Burundi, east Africa. *Epilepsia* **2003**, *44* (7), 950-5.
8. Mwidunda, S. A., et al., A school based cluster randomised health education intervention trial for improving knowledge and attitudes related to *Taenia solium* cysticercosis and taeniasis in Mbulu district, northern Tanzania. *PloS one* **2015**, *10* (2), e0118541.
9. Zammarchi, L., et al., Epidemiology and management of cysticercosis and *Taenia solium* taeniasis in Europe, systematic review 1990-2011. *PloS one* **2013**, *8* (7), e69537.
10. WHO In Resolutions and Decisions Annexes, Control of neurocysticercosis, , Fifty Sixth World Health Assembly, Geneva, Geneva, 2003.
11. Despommier, D. D., Tapeworm infection--the long and the short of it. *N Engl J Med* **1992**, *327* (10), 727-8.
12. Richards, F. O., et al., Cysticercosis in Los Angeles County. *JAMA* **1985**, *254* (24), 3444-8.
13. DeGiorgio, C., et al., Sero-prevalence of *Taenia solium* cysticercosis and *Taenia solium* taeniasis in California, USA. *Acta neurologica Scandinavica* **2005**, *111* (2), 84-8.
14. Cantey, P. T., et al., Neglected parasitic infections in the United States: cysticercosis. *Am J Trop Med Hyg* **2014**, *90* (5), 805-9.
15. Hotez, P. J., Neglected infections of poverty in the United States of America. *PLoS Negl Trop Dis* **2008**, *2* (6), e256.
16. Fleury, A., et al., *Taenia solium*: Development of an Experimental Model of Porcine Neurocysticercosis. *PLoS Negl Trop Dis* **2015**, *9* (8), e0003980.
17. WHO, Accelerating work to overcome the global impact of neglected tropical diseases A roadmap for implementation. Geneva, 2012. Available from: whqlibdoc.who.int/hq/2012/WHO_HTM_NTD_20121_eng.pdf **2012**.
18. Hartmann, P. J., Miscellanea curiosa sive ephemeridium medicophysicarum Germanicarum Academiae Imperialis Leopoldinae naturae curiosorum decuria II. *Observatio 34 Literis Joannis Ernesti Adelbuneri Nurnberg pp* 1688, 58-59.
19. Kuchenmeister, F., The *Cysticercus cellulosus* transformed within the organism of man into *Taenia solium*. *Lancet* **1861**, *i*, 39.
20. Kuchenmeister, F., Experimenteller nachweis, dass *Cysticercus cellulosae* innerhalb des menschlichen darmkanales sich in *Taenia solium* umwandelt. *Wiener Medizinische Wochenschrift* **1855**, *5*, 1-4.
21. Merchant, M. T., et al., *Taenia solium*: description of the intestinal implantation sites in experimental hamster infections. *J Parasitol* **1998**, *84* (4), 681-5.
22. Camacho, S. D., et al., Serology as an indicator of *Taenia solium* tapeworm infections in a rural community in Mexico. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1990**, *84* (4), 563-566.
23. de Aluja, A. S., et al., Experimental *Taenia solium* cysticercosis in pigs: characteristics of the infection and antibody response. *Vet Parasitol* **1996**, *61* (1-2), 49-59.
24. Rodriguez-Contreras, D.; Skelly, P. J., Molecular and functional characterization and tissue localization of 2 glucose transporter homologues (TGTP1 and TGTP2) from the tapeworm *Taenia*

- solium*. *Parasitology* **1998**, *117*, 579-88.
25. Sciutto, E., et al., Development of the S3Pvac vaccine against porcine *Taenia solium* cysticercosis: a historical review. *J Parasitol* **2013**, *99* (4), 686-92.
26. Gauci, C., et al., Vaccine development against the *Taenia solium* parasite: the role of recombinant protein expression in *Escherichia coli*. *Bioengineered* **2013**, *4* (5), 343-7.
27. Silver, S. A., et al., Cerebral cysticercosis mimicking malignant glioma: a case report. *Acta cytologica* **1996**, *40* (2), 351-7.
28. White, A. C., Jr., et al., *Taenia solium* cysticercosis: host-parasite interactions and the immune response. *Chem Immunol* **1997**, *66*, 209-30.
29. Leid, R. W., et al., Parasite defense mechanisms for evasion of host attack; a review. *Vet Parasitol* **1987**, *25* (2), 147-62.
30. Laclette, J. P., et al., Paramyosin is the *Schistosoma mansoni* (Trematoda) homologue of antigen B from *Taenia solium* (Cestoda). *Molecular and biochemical parasitology* **1991**, *44* (2), 287-95.
31. Laclette, J. P., et al., Paramyosin inhibits complement C1. *J Immunol* **1992**, *148* (1), 124-8.
32. Restrepo, B. I., et al., Analysis of immune lesions in neurocysticercosis patients: central nervous system response to helminth appears Th1-like instead of Th2. *Journal of neuroimmunology* **1998**, *89* (1-2), 64-72.
33. Lightowers, M. W., Eradication of *Taenia solium* cysticercosis: a role for vaccination of pigs. *International journal for parasitology* **1999**, *29* (6), 811-7.
34. Song, E. K., et al., Unusual manifestations of *Taenia solium* infestation. *Journal of gastroenterology* **2004**, *39* (3), 288-91.
35. Del Giudice, P., et al., Subcutaneous cysticercosis. *Annales de dermatologie et de venerologie* **1996**, *123* (8), 474-7.
36. Salgado, P., et al., Cysticercosis. Clinical classification based on imaging studies. *Archives of internal medicine* **1997**, *157* (17), 1991-7.
37. Fleury, A., et al., Immunopathology in *Taenia solium* neurocysticercosis. *Parasite Immunol* **2016**, *38* (3), 147-57.
38. Mitchell, W. G.; Crawford, T. O., Intraparenchymal cerebral cysticercosis in children: diagnosis and treatment. *Pediatrics* **1988**, *82* (1), 76-82.
39. Nash, T. E.; Patronas, N. J., Edema associated with calcified lesions in neurocysticercosis. *Neurology* **1999**, *53* (4), 777-81.
40. Dorny, P., et al., Immunodiagnostic tools for human and porcine cysticercosis. *Acta tropica* **2003**, *87* (1), 79-86.
41. Ide, L., To differentiate *Taenia* eggs. *J Clin Microbiol* **2012**, *50* (8), 2836; author reply 2837.
42. Jimenez, J. A., et al., Differentiating *Taenia* eggs found in human stools: does Ziehl-Neelsen staining help? *Trop Med Int Health* **2010**, *15* (9), 1077-81.
43. Chapman, A., et al., Isolation and characterization of species-specific DNA probes from *Taenia solium* and *Taenia saginata* and their use in an egg detection assay. *Journal of clinical microbiology* **1995**, *33* (5), 1283-8.
44. Praet, N., et al., Bayesian modelling to estimate the test characteristics of coprology, coproantigen ELISA and a novel real-time PCR for the diagnosis of taeniasis. *Trop Med Int Health* **2013**, *18* (5), 608-14.
45. Yamasaki, H., et al., DNA differential diagnosis of taeniasis and cysticercosis by multiplex PCR. *J Clin Microbiol* **2004**, *42* (2), 548-53.
46. Yamasaki, H., et al., DNA differential diagnosis of taeniasis and cysticercosis by multiplex PCR. *Journal of clinical microbiology* **2004**, *42* (2), 548-53.
47. Jeri, C., et al., Species identification after treatment for human taeniasis. *Lancet* **2004**, *363* (9413), 949-50.
48. Allan, J. C., et al., Immunodiagnosis of taeniasis by coproantigen detection. *Parasitology* **1990**, *101 Pt 3*, 473-7.
49. Wilkins, P. P., et al., Development of a serologic assay to detect *Taenia solium* taeniasis. *Am J Trop Med Hyg* **1999**, *60* (2), 199-204.
50. Leite, C. C., et al., MR imaging of intramedullary and intradural-extramedullary spinal

- cysticercosis. *AJR Am J Roentgenol* **1997**, 169 (6), 1713-7.
51. Tsang, V. C., et al., An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). *J Infect Dis* **1989**, 159 (1), 50-9.
 52. Proano-Narvaez, J. V., et al., Laboratory diagnosis of human neurocysticercosis: double-blind comparison of enzyme-linked immunosorbent assay and electroimmunotransfer blot assay. *Microbiol* **2002**, 40 2115-18.
 53. Gekeler, F., et al., Sensitivity and specificity of ELISA and immunoblot for diagnosing neurocysticercosis. *Eur J Clin Microbiol Infect Dis* **2002**, 21 (3), 227-9.
 54. White, A. C., Jr., et al., Diagnosis and Treatment of Neurocysticercosis: 2017 Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). *Clin Infect Dis* **2018**, 66 (8), 1159-1163.
 55. Richards, F.; Schantz, P. M., Treatment of *Taenia solium* infections. *Lancet (London, England)* **1985**, 1 (8440), 1264-5.
 56. The-Medical-Letter, The medical letter handbook of adverse drug interactions. 2000.
 57. Keeling, J. E. D., The chemotherapy of cestode infections. *Adv Chemother* **1978**, 3, 109-152.
 58. Del Brutto, O. H., Neurocysticercosis. *Continuum (Minneap Minn)* **2012**, 18 (6 Infectious Disease), 1392-416.
 59. Sotelo, J., et al., Cysticercosis. *Current clinical topics in infectious diseases* **1996**, 16, 240-59.
 60. Bittencourt, P. R., et al., Phenytoin and carbamazepine decreased oral bioavailability of praziquantel. *Neurology* **1992**, 42 (3 Pt 1), 492-6.
 61. Garcia, H. H., et al., Cysticidal Efficacy of Combined Treatment With Praziquantel and Albendazole for Parenchymal Brain Cysticercosis. *Clin Infect Dis* **2016**, 62 (11), 1375-9.
 62. Sotelo, J.; Jung, H., Pharmacokinetic optimisation of the treatment of neurocysticercosis. *Clinical pharmacokinetics* **1998**, 34 (6), 503-15.
 63. Lara-Aguilera, R., et al., *Taenia solium* taeniasis and neurocysticercosis in a Mexican rural family. *The American journal of tropical medicine and hygiene* **1992**, 46 (1), 85-8.
 64. Schantz, P. M., et al., Neurocysticercosis in an Orthodox Jewish community in New York City. *N Engl J Med* **1992**, 327 (10), 692-5.
 65. Fan, P. C., et al., Survival of *Taenia solium* cysticerci in carcasses of pigs kept at 4 C. *The Journal of parasitology* **1998**, 84 (1), 174-5.
 66. Carabin, H.; Traore, A. A., taeniasis and cysticercosis control and elimination through community-based interventions. *Curr Trop Med Rep* **2014**, 1 (4), 181-193.
 67. O'Neal, S. E., et al., Ring-screening to control endemic transmission of *Taenia solium*. *PLoS Negl Trop Dis* **2014**, 8 (9), e3125.
 68. Organization, W. H., Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva. Licence: CC BY-NC-SA 3.0 IGO **2020**.
 69. Ouma, E., et al., Demand for *Taenia solium* Cysticercosis Vaccine: Lessons and Insights From the Pig Production and Trading Nodes of the Uganda Pig Value Chain. *Front Vet Sci* **2021**, 8, 611166.
 70. Manoutcharian, K., et al., Cysticercosis: identification and cloning of protective recombinant antigens. *J Parasitol* **1996**, 82 (2), 250-4.
 71. Lightowlers, M. W.; Gauci, C. G., Vaccines against cysticercosis and hydatidosis. *Vet Parasitol* **2001**, 101 (3-4), 337-52.
 72. Molinari, J. L., et al., Field trial for reducing porcine *Taenia solium* cysticercosis in Mexico by systematic vaccination of pigs. *Vet Parasitol* **1997**, 69 (1-2), 55-63.
 73. Gonzales, A. E., et al., Effective, single-dose treatment of porcine cysticercosis with oxfendazole. *Am J Trop Med Hyg* **1996**, 54 (4), 391-4.
 74. D'Souza, P. E.; Hafeez, M., Detection of *Taenia solium* cysticercosis in pigs by ELISA with an excretory-secretory antigen. *Vet Res Commun* **1999**, 23 (5), 293-8.
 75. Theis, J. H., et al., DNA-confirmed *Taenia solium* cysticercosis in black bears (*Ursus americanus*) from California. *The American journal of tropical medicine and hygiene* **1996**, 55 (4), 456-8.

30. *Diphyllobothrium latum*

(Linnaeus 1758)

Pronunciation: \dī-fil-ə-bäth-rē-əm\\lä-t-əm\

Introduction

Diphyllobothrium latum (\DYE-fil-ow-both-REE-um\\la-tum\), the fish tapeworm, is a member of the order Pseudophyllidea and can grow to a length of 15 meters. It has achieved a recorded length of 25 meters, making it the longest parasite to infect humans.¹ As many as 20 million people may be infected worldwide by fish tapeworms; mainly *D. latum* (\la-tum\), but also *D. pacificum* (\pa-sif-ik-um\), *D. nihonkaiense* (Nee-hon-keye-en-see\), *D. cordatum* (\core-dah-tum\), *D. ursi* (\ur-see\), *D. dendriticum* (\DEN-drit-ik-um\, \den-'dri-tik-əm\), *D. lanceolatum* (\LAN-see-o-LATE-um\, \lan-sē-ə-,lä-t-əm\), *D. dalliae* (\DAL-ee-uh\), and *D. yonagoen-*

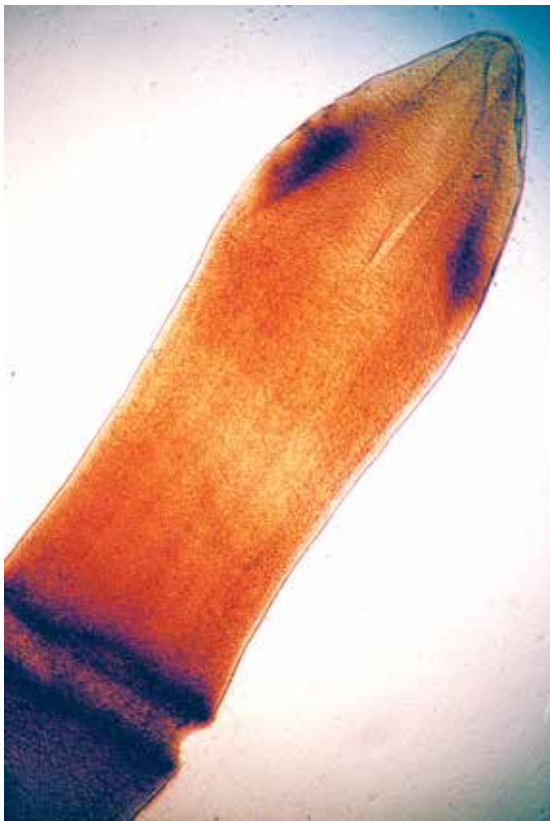


Figure 30.1. The plerocercoid stage of *D. latum*. This is the infective stage for the definitive host.



Figure 30.2. The scolex and mature proglottids of *D. latum*. Courtesy H. Zaiman.

sis (\Yon-uh-go-en-sus\).^{2,3} *D. latum* can grow at a rate of 22 cm/day, releasing 1,000,000 eggs daily, and live for up to 25 years.^{4,6} It is acquired by eating raw or undercooked fish. Its proglottids are wider than long, so the worm is often referred to as the broad tapeworm. As with all other adult tapeworms, it lives in the lumen of the small intestine and usually does little harm to its host. It has a

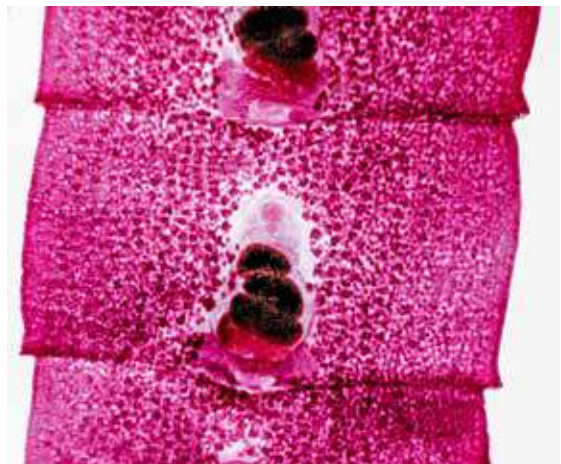
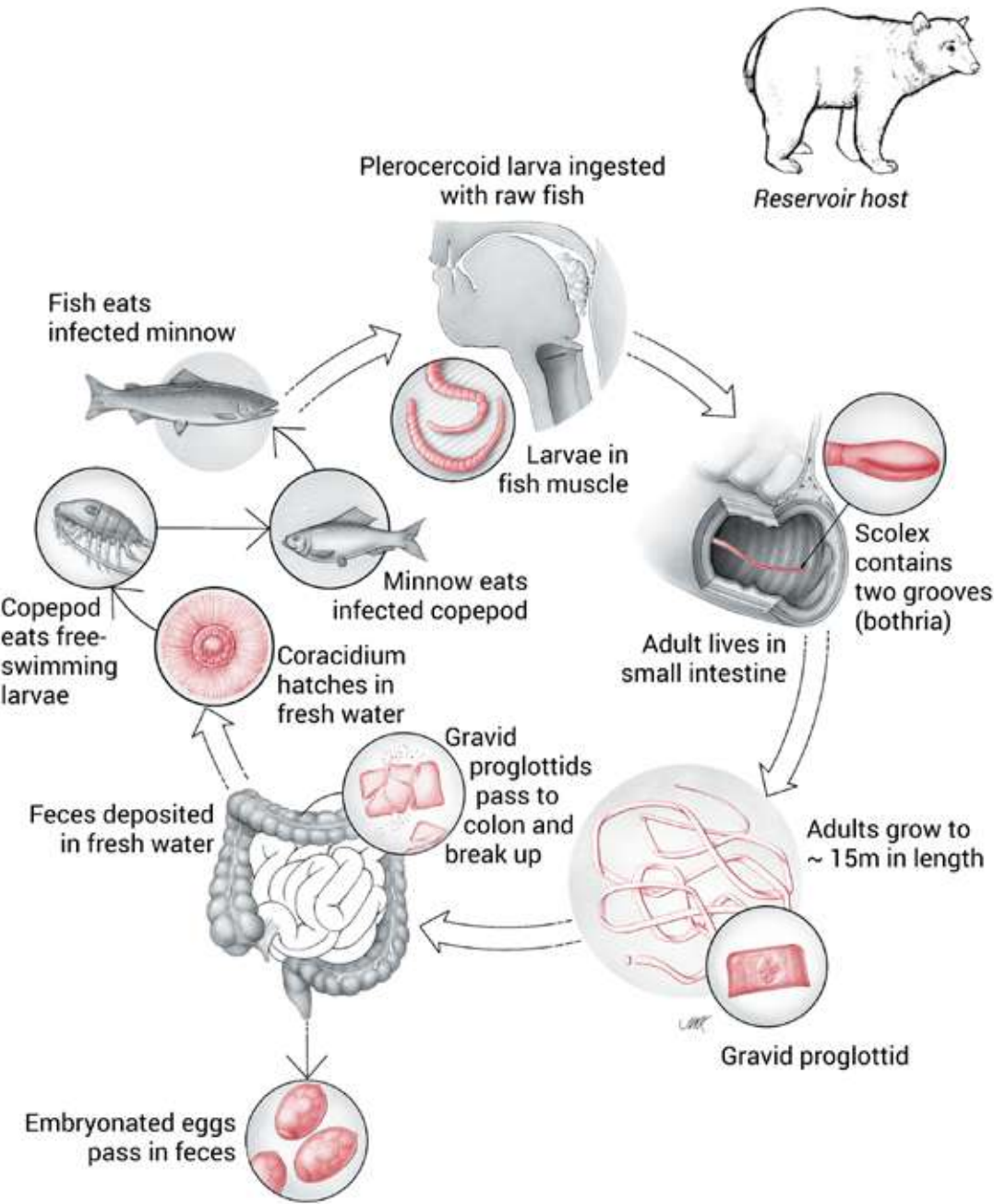


Figure 30.3. Mature proglottids of *D. latum*.

Diphyllobothrium latum



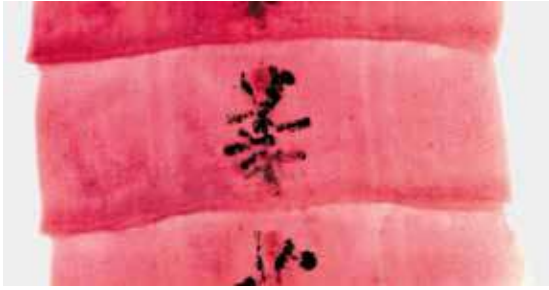


Figure 30.4. Gravid proglottids of *D. latum*.



Figure 30.5. Unembryonated egg of *D. latum*. 65 μm x 45 μm .



Figure 30.6. Hatching egg of *D. latum*. Note operculum.

unique affinity for absorbing vitamin B₁₂, and as a result, the infection can have pathological consequences for some infected individuals.

All species in this group have similar complex life cycles. In most cases, they use invertebrates (e.g., copepods), and anadromous (e.g., wild salmon) and freshwater fishes as intermediate hosts. Most carnivores are susceptible to infection with *D. latum*, includ-



Figure 30.7. Ciliated coracidium. 60 μm in diameter.

ing dog, bear, cat, fox, martin, mink, and other wild mammals. Some of these hosts are important reservoirs for human infection.

D. latum infection is common throughout Scandinavia, though prevalence in that region has decreased due, in large part, to vastly improved sanitation.^{7,8} The tapeworm was probably introduced into North America (especially in the Great Lakes and Lake Winnipeg, Manitoba) by northern European immigrants.⁹ Infections are reported regularly in Russia, Brazil, and Japan, although infections have also been reported in Asia, Europe, and the Americas.⁹

The prevalence of *D. latum* infection is declining in North America due to modern sanitation practices. *D. nihonkaiense* infection in humans has been reported in the state of Washington.^{10,11} A related sub-species, *D. latum* var. *parvum* infects people in Korea and China.¹² In circumpolar regions, infection due to *D. dendriticum* is common in both people and animals.¹³ *Diphyllobothrium pacificum* (also known as *Adenocephalus pacificus* (\a-deno-SEF-a-lus\,\ə-'de-nə-'sefəls\)), has been reported from Peru, Chile, and Ecuador, as well as Australia and may be an important species in these regions (possibly linked to

the consumption of ceviche).^{14, 15}

Historical Information

Descriptions of infection due to *D. latum* go back thousands of years, with archaeological evidence of human infection in Peru from 10,000–4,000 BCE.¹⁶ In 1609, the Swiss physician Félix Plater observed and reported that *D. latum* infected humans.¹⁷ In 1751, Carl Linnaeus classified *D. latum*.¹⁸ In 1917, Constantine Janicki and Felix Rosen, in a series of elegant epidemiological observations and laboratory experiments, described and illustrated its complex life cycle.¹⁹

Life Cycle

Infection begins when undercooked or raw fish, infected with the plerocercoid metacystode of the parasite, is eaten (Fig. 30.1). In the northern hemisphere, pike and percids are the most common source of infection in many regions of the world.⁹ After ingestion, the parasite is released from the fish's flesh in the stomach. The plerocercoid larvae, now free of fish muscle, pass to the small intestine and attach to the intestinal wall by applying their two bothria (grooves) to the epithelial surface. They grow and develop into adult worms (Fig. 30.2), becoming fully formed strobilae within three months. In human infection, eggs begin to pass into the stool

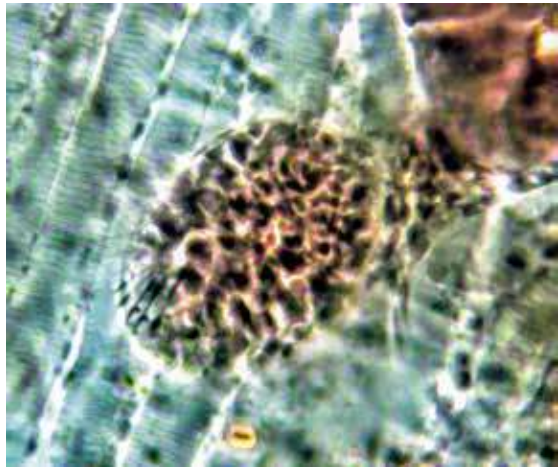


Figure 30.9. Proceroid stage of *D. latum* in tissues of *Diaptomus* spp. Note hooklets. (Phase-contrast).

15–45 days after ingesting the infected fish.⁶ Usually, only one tapeworm of *D. latum* infects a given individual, but patients can harbor multiple infections. The adult fish tapeworm usually has a life span of 4.5 years but can live for as long as 25 years.⁹

Proglottids (segments) are greater in width than length (Figs. 30.3, 30.4), and contain both sets of sex organs. Proglottids fertilize eggs in adjacent segments. As fertilized proglottids become gravid, eggs (Fig. 30.5) exit from the centrally located uterine pore, entering the lumen of the small intestine. Gravid segments can also break off from the strobila and disintegrate in the small intestine. The fertilized, unembryonated eggs pass out of the host with the feces and must be deposited in freshwater if the life cycle is to continue.

The eggs measure 70 μm and embryonate over a 9–12 day period before hatching (Fig. 30.6). The motile coracidium (Fig. 30.7) (ciliated oncosphere) emerges from the egg and immediately begins to swim. Free-living coracidia can live for 3–4 days before exhausting their stored food reserves. Motile coracidia attract the attention of zooplankton crustacean predators (e.g., *Cyclops* spp. and *Diaptomus* spp.) (Fig. 30.8) and become ingested. Instead of being digested by the crustacean



Figure 30.8. *Diaptomus* spp. infected with *D. latum*.

intermediate host, the coracidium burrows into the body cavity and develops into an immature metacestode called a procercoid (Fig. 30.9).

When infected crustaceans are consumed by small freshwater fish and salmon parr, the procercoid is freed from the crustacean and penetrates the wall of the small intestine of the fish, eventually lodging in the muscles, or various viscera, such as the liver and gonads. It then differentiates and grows into a plerocercoid metacestode, the infective stage for humans and other mammals. If the second intermediate host dies before being ingested, the plerocercoids in its viscera are stimulated to migrate to the muscles.⁶

When infected minnows are eaten by large predatory fish species, such as members of the perch, pike, and salmon families, plerocercoids can be transferred to the body muscles of these larger fish. Carnivores, including humans, often consume these larger fish, and a resulting intestinal tapeworm infection can then develop.

Cellular and Molecular Pathogenesis

Pseudophyllid tapeworms absorb large quantities of vitamin B12 and their analogues. They employ a tegumental cyanocobalamin receptor that has a high affinity for several analogs of this compound, including cobalamin, and mediate dissociation of the vitamin B12-intrinsic factor complex.²⁰ Cobalamin is converted to adenosyl-cobalamin, a coenzyme for methyl-malonyl-CoA mutase. Anaerobic energy metabolism relies on propionate production, and these two enzymes are integral to that metabolic pathway.²¹ The fish tapeworm can accumulate B12 at a rate 100 times faster than the infected host.⁶ Almost half of all patients infected with *D.*

latum exhibit decreased B12 levels, but only a minority develop clinically apparent anemia. Host factors, the number of infecting worms, and the specific tapeworm involved determine the risk of developing B12 deficiency and macrocytic hypochromic anemia.³

Clinical Disease

In most individuals, infection with the fish tapeworm results in no obvious symptoms. Infections with multiple worms may cause nonspecific symptoms such as watery diarrhea, fatigue, and rarely mechanical obstruction of the small bowel.^{10, 22, 23} Exhaustion of vitamin B12 is a slow process, taking many months to years.²⁴ The full picture of megaloblastic anemia due to B12 deficiency is indistinguishable from that due to other causes. While up to 40% of infected individuals will have B12 deficiency, less than 2% of infected individuals develop megaloblastic anemia.²⁵ The reason for the relative infrequency of megaloblastic anemia among most of those infected with *D. latum* needs to be better understood. Host genetic factors may predispose certain infected individuals to suffer the full effect of this deficiency. One study showed that a few *D. latum* patients suffering from megaloblastic anemia had less intrinsic factor than those who were free of anemia, but who also harbored the same tapeworm species. Other studies have not confirmed this observation.²⁵ Eosinophilia has been reported in a number of cases.^{23, 26}

Diagnosis (see Clinical Appendix)

Segments of worm in stool (Figs. 30.3, 30.4) sometimes alert patients that they harbor a tapeworm, but most proglottids break up in the intestinal tract before exiting the host. Diagnosis is typically made by microscopic identification of non-embryonated eggs (Figs. 30.5, C.58.) in stool. Molecular tests

are available for diagnosis, allowing for species determination, but most diagnosis is done using visual microscopy.⁶

Treatment (see Clinical Appendix)

The drug of choice is praziquantel (5–10 mg/kg once).²⁷ Niclosamide is an alternative therapy that appears to be effective.⁶

Prevention and Control

In sylvatic settings, numerous reservoir hosts sustain the life cycle. Interference with this phase of its ecology would be difficult, even if possible. On an individual basis, the best way to avoid infection with *D. latum* is to avoid eating freshwater fish unless it is well-cooked or has been previously frozen. Sushi is predominantly made from

saltwater fish species that do not acquire *D. latum* or related tapeworm species. A few cases have been reported from eating sushi prepared from Pacific coast salmon, which spend a portion of their life in freshwater.²⁸

The culinary habit among many Jewish mothers or grandmothers of teaching their daughters to prepare gefilte fish by tasting the raw mixture led many a female to acquire this infection in the United States, and was popularized in a medical anthropological description by Robert S. Desowitz.²⁹ Today, proper disposal of human feces in the Great Lakes region of the United States has dramatically reduced the prevalence of *D. latum*. In Scandinavian countries, gravlax and various other marinated raw fish dishes remain sources of infection for this tapeworm.

References

1. von Bonsdorff, B., *Diphyllbothriasis in man*. Academic Press, New York, NY. 1977.
2. Chai, J. Y., et al., Fish-borne parasitic zoonoses: status and issues. *Int J Parasitol* **2005**, 35 (11-12), 1233-54.
3. Jimenez, J. A., et al., *Diphyllbothrium pacificum* infection is seldom associated with megaloblastic anemia. *Am J Trop Med Hyg* **2012**, 87 (5), 897-901.
4. Kuhlow, F., [Studies on the development of *Diphyllbothrium latum*]. *Z Tropenmed Parasitol* **1955**, 6 (2), 213-25.
5. Dogiel, V. A., Allgemeine Parasitologie. *Parasitol. Schriftenr.* **1963**, 16, 1-523.
6. Scholz, T., et al., Update on the human broad tapeworm (genus *Diphyllbothrium*), including clinical relevance. *Clin Microbiol Rev* **2009**, 22 (1), 146-60, Table of Contents.
7. Kyronseppa, H.; J., The occurrence of human intestinal parasites in Finland. *Scand Dis* **1993**, 25 (5), 671-3.
8. Dick, T. A., et al., Diphyllbothriasis: update on human cases, foci, patterns and sources of human infections and future considerations. *Southeast Asian J Trop Med Public Health* **2001**, 32 Suppl 2, 59-76.
9. Dick, T. A., et al., Diphyllbothriasis: update on human cases, foci, patterns and sources of human infections and future considerations. *The Southeast Asian journal of tropical medicine and public health* **2001**, 32 Suppl 2, 59-76.
10. Shimizu, T., et al., *Diphyllbothrium nihonkaiense* infection linked to chilled salmon consumption. *BMJ Case Rep* **2012**, 2012.
11. Torres, P., et al., [New records of human diphyllbothriasis in Chile (1981-1992), with a case of multiple *Diphyllbothrium latum* infection]. *Boletin chileno de parasitologia* **1993**, 48 (3-4), 39-43.
12. Lee, S. H., et al., Two rare cases of *Diphyllbothrium latum parvum* type infection in Korea. *The Korean journal of parasitology* **1994**, 32 (2), 117-20.

13. Curtis, M. A.; Bylund, G., *Diphyllobothriasis*: fish tapeworm disease in the circumpolar north. *Arctic medical research* **1991**, 50 (1), 18-24.
14. Kuchta, R., et al., Pacific Broad Tapeworm *Adenocephalus pacificus* as a Causative Agent of Globally Reemerging Diphyllobothriosis. *Emerg Infect Dis* **2015**, 21 (10), 1697-703.
15. Moore, C. V., et al., Rare Human Infection with pacific broad tapeworm *Adenocephalus pacificus*, Australia. *Emerg Infect Dis* **2016**, 22 (8), 1510-2.
16. Reinhard, K. J.; Barnum, S. V., Parasitology as an interpretative tool in archaeology. *Am. Antiq* **1991**, 57, 231-245.
17. Plater, F., Praxeos seu de cognoscendis, praedicendis praecudensis curandis affectibus homini incommodantibus tractatus tertius et ultimus. *De vitiis libris duobus agens quorum primum corporis secundus Exretorum vitia continet Typis Conradi Waldkirchii* **1609**.
18. Linnaeus, C., *Taenia osculis lateralibus solitaris. Amoenitates Academicae Holmiae Laurentium Salvium* 2, 80-81.
19. Janicki, C., Le cycle evolutif du *Dibothriocephalus latus* L. *Soc Neuchateloise Sci Natur Bull* **1917**, 42, 19-53.
20. Friedman, P. A., et al., Characterization of cobalamin receptor sites in brush-border plasma membranes of the tapeworm *Spirometra mansonioides*. *The Journal of biological chemistry* **1983**, 258 (7), 4261-5.
21. Smyth, J. D.; McManus, D. P., *The Physiology and Biochemistry of Tapeworms*. 1989.
22. Lee, S. H., et al., *Diphyllobothrium latum* infection in a child with recurrent abdominal pain. *Korean J Pediatr* **2015**, 58 (11), 451-3.
23. Nawa, Y., et al., Sushi delights and parasites: the risk of fishborne and foodborne parasitic zoonoses in Asia. *Clin Infect Dis* **2005**, 41 (9), 1297-303.
24. Goodman, K. I.; Salt, W. B., Vitamin B12 deficiency. Important new concepts in recognition. *Postgraduate medicine* **1990**, 88 (3), 147-50, 153.
25. Saarni, M., et al., Symptoms in carriers of *Diphyllobothrium latum* and in uninfected controls. *Acta Med Scand* **1963**, 173, 147-154.
26. Arizono, N., et al., Diphyllobothriasis associated with eating raw pacific salmon. *Emerg Infect Dis* **2009**, 15 (6), 866-70.
27. Groll, E., Praziquantel for cestode infections in man. *Acta Trop* **1980**, 37 (3), 293-6.
28. Hutchinson, J. W., et al., Diphyllobothriasis after eating raw salmon. *Hawaii medical journal* **1997**, 56 (7), 176-7.
29. Desowitz, R. S.; A., *New Guinea Tapeworms and Jewish Grandmothers: of Parasites and People. W W Norton Co Pubs* **1981**.



James Paget, M.D. (1814–1899)

Paget observed the first parasitic worm infection of humans to be identified by microscopy. He did so while attending medical school at St. Bartholomew's Hospital in London. The parasite was *Trichinella spiralis* and was seen in a piece of muscle tissue obtained at autopsy from a 51-year-old bricklayer who had died of tuberculosis. Paget went on to become a famous pathologist. High among his other accomplishments, he described the pathological features of the bone disease that bears his name.

31. Other Tapeworms of Medical Importance

Hymenolepis nana

(Siebold 1852)

Pronunciation: \hī-mə-'nāl-ə-pəs\|na-nə\

Introduction

Hymenolepis nana (\HI-men-OL-eh-pis\|Na-nuh), is a member of the order Cyclophyllidea, has a worldwide distribution, and infects mostly children. Prevalence in children as high as 25% has been reported in certain areas.^{1,3} *H. nana* infection is common among children living in poor neighborhoods and institutional settings.^{4,5} As its species name implies, this is a small tapeworm, measuring 34–45 mm in length. The adult consists of 150–200 proglottids and lives in the lumen of the small intestine, loosely attached to the epithelial cells of the villi. Its scolex has four suckers and a single row of hooklets. Rodents are significant reservoir hosts for this parasite. Like *Strongyloides stercoralis*, *H. nana* can complete its entire life cycle within the human host. Autoinfection results in a high worm burden, particularly in immunosuppressed patients.^{6,7} In 2015, malignant transformation and metastasis of cells from *H. nana* in an HIV-1 infected individual was described.⁸

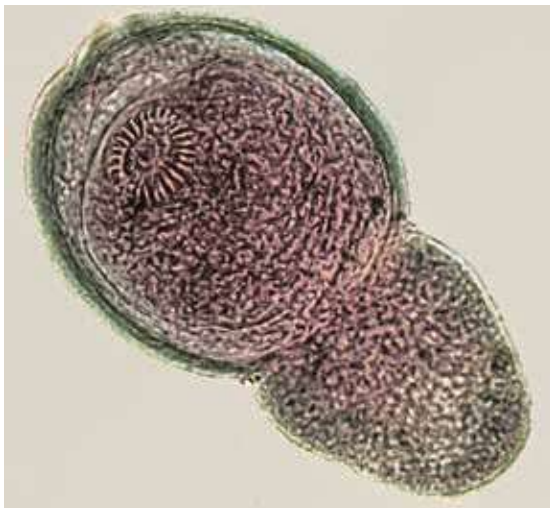


Figure 31.1. The cysticercoid of *Hymenolepis nana*. 350 μ m x 200 μ m.



Figure 31.2. Egg of *Hymenolepis nana*. 35 μ m x 40 μ m.

A second related species, *Hymenolepis microstoma*, has been described as a secondary infection in *H. nana*-infected patients from remote communities in Western Australia.⁹

Historical Information

In 1852, Theodore Bilharz identified *H. nana* when he discovered it on autopsy of a six-year-old boy who died of meningitis, and whose small intestine harbored numerous adult parasites.¹⁰ In 1887, Giovanni Grassi demonstrated that *H. nana* could have a direct transmission cycle in rats without an intermediate host.¹¹ In 1911, Charles Nicolle and Edward A. Minchin demonstrated that *H. nana* can also have an indirect transmission cycle involving fleas or beetles as intermediate hosts.¹² In 1921, Y. Saeki determined that *H. nana* could have a direct transmission cycle in humans.

Life Cycle

Infection can begin in one of two ways: by ingesting the cysticercoid metacestode (Fig. 31.1) along with an infected insect, or by ingesting embryonated eggs. Infective stages

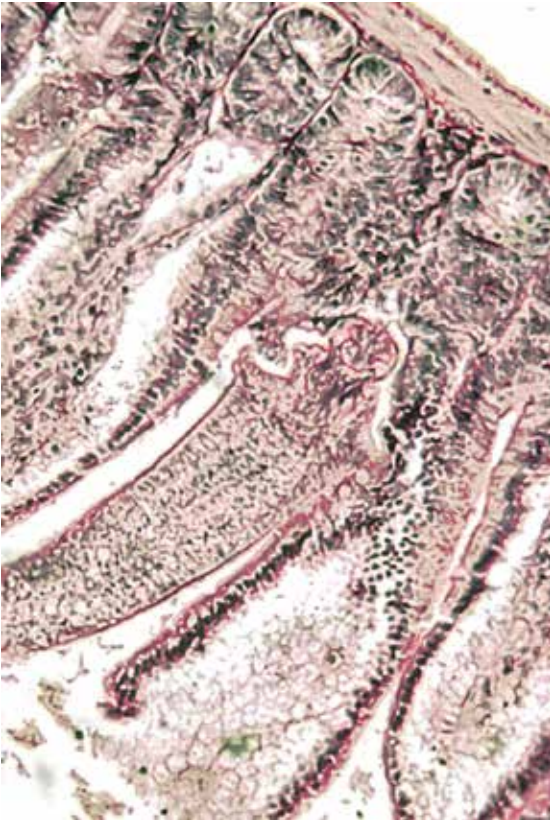


Figure 31.3. Histological section of *H. nana*, in situ.

of *H. nana* are sometimes present in *Tenebrio* larvae (i.e., mealworms often found contaminating cereals and grains), or in rat feces. If eggs are ingested (Fig 31.2), the oncospheres hatch in the small intestine and penetrate the lamina propria of a villus. There, each larva differentiates into the cysticercoid (juvenile stage). This stage reenters the intestinal lumen and attaches to the surface of the villous tissue (Fig. 31.3), where it rapidly differentiates into an immature adult parasite with four suckers and a single row of hooklets (Fig. 31.4).

H. nana grows to full maturity within a three to four-week period. If the cysticercoid is ingested, then it attaches to the wall of the small intestine and differentiates and matures to the adult worm, usually within a two-week period. Although the lifespan of an adult worm is only 4–6 weeks, the cycle of autoinfection can allow an infection to last for years.¹³

Mating between adjacent proglottids (Fig.

31.5) produces hundreds of fertilized eggs. Gravid segments break off from the strobila and disintegrate in the small intestine, releasing the fertilized, embryonated eggs. Autoinfection, with released eggs hatching directly within the intestine, is possible but rarely occurs, as immunity to reinfection develops in most instances.¹⁴ Eggs deposited in the feces may be ingested by the larvae of beetles, by rodents, or by humans. In the invertebrate host, the oncospheres hatch, penetrate the gut, and enter the insects' hemocoel, where they differentiate into cysticercoid metacestodes.

Cellular and Molecular Pathogenesis

Infection is usually self-limited in adult patients but not in very young children, probably reflecting the age-specificity of the development of protective immunity. The cysticercoid stage is relatively non-



Figure 31.4. Scolex of *Hymenolepis nana*. It has four suckers as well as hooks.

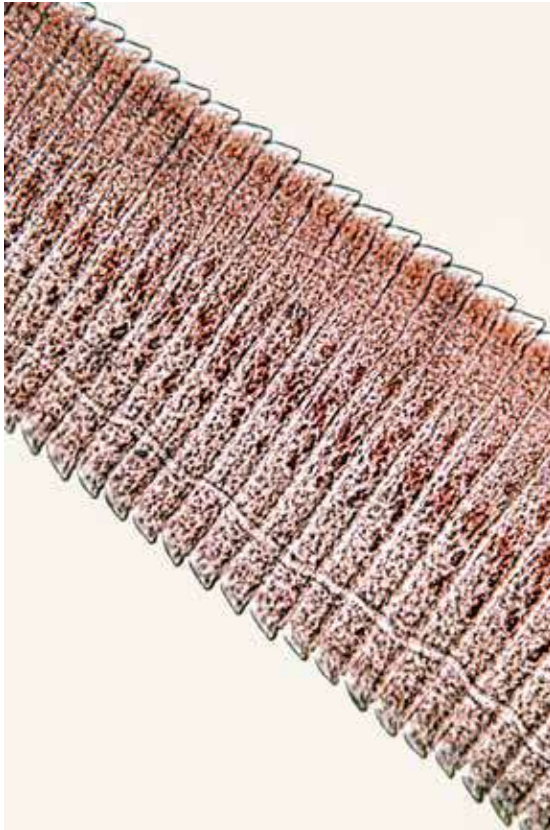


Figure 31.5. Mature proglottids of *Hymenolepis nana*. 400 μ m wide.

immunogenic, allowing for autoinfection to develop. In contrast, infection initiated by ingesting the egg stage triggers a rapid and robust protective immune response.^{15,16} A low but detectable, protective humoral immune response occurs due to exposure to the entire life cycle and is transferable to a naive host.^{1,17} In experimental infections of mice, the cysticercoid attracts eosinophils by secreting factors into the local area of infection, especially during reinfection, and these host cells may play a role in preventing the establishment of new infections with *H. nana*.^{18,19} Regardless of the immunological background of the mouse strain, INF- γ is always a dominant feature of their response to infection, and expulsion of worms may be due directly to the upregulation of this peptide. Antibodies of the IgE class may also play a role in protection.^{20,21} Immunity to *H. nana* is multifactorial, involving both Th1 and Th2 responses.¹⁷

Clinical Disease

Most infections are benign. Heavy infections are accompanied by diarrhea.⁵ It is unclear whether *H. nana* causes symptoms such as abdominal pain, headache, and itching around the anus or if these complaints are due to co-infection with other pathogens.

Diagnosis (see Clinical Appendix)

Microscopic identification of embryonated eggs (Fig. 32.2) in the stool is the definitive diagnosis. When whole pieces of strobila are passed, they can be identified directly, or the eggs can be expressed from gravid proglottids and then identified.

Treatment (see Clinical Appendix)

Praziquantel is the drug of choice because it affects both the cysticercoid in the villus tissue and the adult.²²⁻²⁵ A single dose of praziquantel (5–10 mg/kg) is recommended. In contrast, niclosamide kills the adult, but it is ineffective against the metacestode.²⁶ If niclosamide is the only drug available, treatment for several days or re-examining the patient's stool after therapy is required because an additional course of therapy may be necessary. Nitazoxanide has been investigated as a broad-spectrum antiparasitic for children with multiple intestinal protozoa and helminths, including *H. nana*, and may be an alternative therapy.²⁷⁻²⁹

Prevention and Control

Preventing contamination of food and water supplies with human feces and infected fleas and beetles is the best approach to controlling *H. nana* infection. In treating individuals, especially small children, it is sometimes difficult to achieve a cure due to autoinfection. Rodent reservoir hosts contaminate the envi-

ronment and, in many situations, controlling their populations has reduced the incidence of infection. Eliminating small rodent populations is challenging. Reintroduction is the norm.

Hymenolepis diminuta

(Rudolphi 1819)

Pronunciation: \hī-mə-'nāl-ə-pəs\\də-mi-nū-tə\

Introduction

Hymenolepis diminuta (\HI-men-OL-oh-pis\ di-min-oo-tuh) is found worldwide and has many reservoir hosts, including dogs, cats, and many species of rodents. As with *H. nana*, it is primarily an infection of children.¹²

Historical Information

In 1819, Karl Asmund Rudolphi described the morphology of *H. diminuta*.³⁰ In 1858, David Weinland described the infection in humans.³¹

Life Cycle

Infection begins when the cysticercoid is ingested by the infected insect. The immature worm attaches to the intestinal wall with four suckers on its scolex. The adult worm matures within 18 days and grows to 50 cm in length. The strobila contains about 1,000 proglottids at any one time.

Gravid proglottids detach from the strobila and disintegrate in the small intestine. Eggs (Fig. 31.6) pass with the feces and must be ingested by an appropriate intermediate host, either the larva of fleas or flour beetles (*Tenebrio* spp.), to continue the life cycle. In contrast to eggs of *H. nana*, *H. diminuta* ova are not infectious for humans. When eggs were experimentally fed to *Tenebrio moli-*



Figure 31.6. Egg of *H. diminuta*. 75 μ m in diameter.

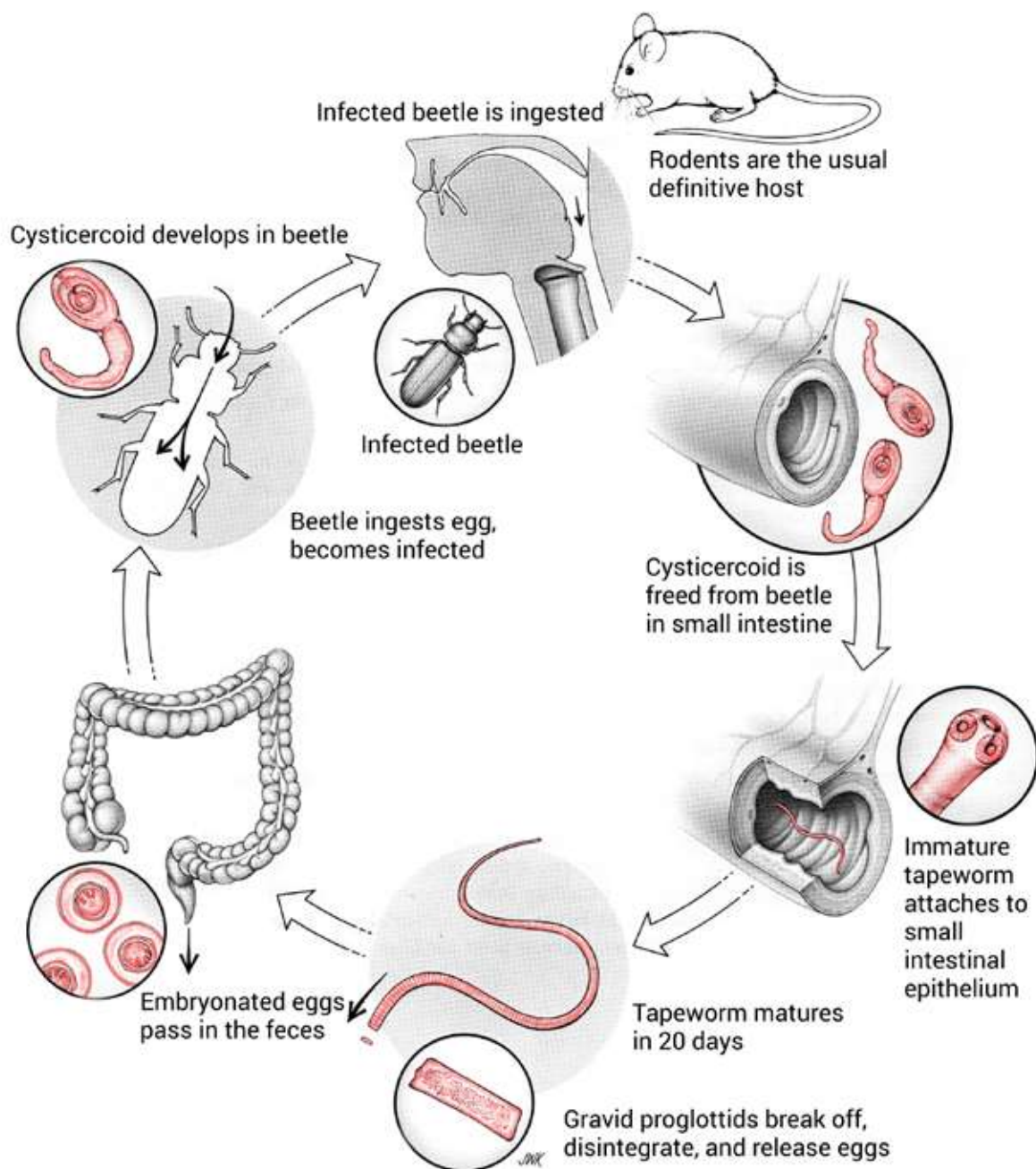
tor (mealworm) larvae, some passed through their gut tract and were incorporated within the fecal pellets. They remained infective for 48 hours, spreading infection among the remaining insect larvae.³²

The egg hatches within the lumen of the insect gut, and the oncosphere penetrates into the hemocoel and develops into the cysticercoid, the infective stage for humans. The life cycle is completed when a human eats an infected insect. Other vertebrates (e.g., rats, mice, and dogs) also serve as definitive hosts. Beetle-to-beetle transmission may be even more significant than cycles involving vertebrate intermediates. It may serve to free this parasite from reliance on the presence of an additional host to complete its life cycle.³²

Cellular and Molecular Pathogenesis

H. diminuta is a well-studied tapeworm, and continues to serve as a model for all adult tapeworms infecting warm-blooded mammals.^{33,34} Despite the wealth of knowledge accumulated on this cestode, little is known of its pathophysiology in humans owing to the rarity with which it infects humans.^{35,36}

Hymenolepis diminuta



Clinical Disease

H. diminuta appears to induce no tissue damage. No clinical symptoms are attributable to this infection, although infections with more than ten worms have been associated with abdominal pain, anorexia, and irritability.³⁷⁻³⁹ In experimental infection in rats, *H. diminuta* has subtle effects on gut transit time and the myoelectric potential, but whether this is the case in human infection has yet to be demonstrated.⁴⁰

Diagnosis (see Clinical Appendix)

Identification of eggs (Figs. 31.6, C.61) in the stool is the definitive method of diagnosis. Occasionally, whole segments of adult worms, which can be identified directly, are also passed in the feces. It is possible to extract eggs from such gravid segments and identify them.

Treatment (see Clinical Appendix)

A single dose of praziquantel is the drug of choice. Niclosamide, given for several days, is an effective alternative drug.³⁵ Adaptation of *H. diminuta* to the golden hamster has created a model laboratory infection for the *in vivo* testing of new anti-cestode drugs.⁴¹

Prevention and Control

H. diminuta, like *H. nana*, must be controlled both in the infected individual and in the reservoir host. The latter is an unrealistic goal in most rural and suburban situations, particularly in less developed countries. Community efforts are aimed at curtailing food contamination, especially whole grains and processed flour, by insects that could harbor the intermediate stage of the worm.

Dipylidium caninum

(Linnaeus 1758)

Pronunciation: \dī-,pī-'lid-ē-əm\\kā-nin-əm\

Introduction

Dipylidium caninum (\DYE-pie-LID-ee-um\\KAY-nin-um) lives in the lumen of the small intestine of dogs, cats, foxes, hyenas, and occasionally humans. The name of the genus is of Greek origin and means “double pore” or “double opening.”

Life Cycle

The infection is acquired by ingesting an infected adult flea, usually *Ctenocephalides canis* or *Ctenocephalides felis* (Fig. 38.23). The digestive enzymes of the host release the cysticercoid from the flea. The scolex (Fig. 31.7) attaches to the villous surface of the small intestine, and within 25 days, the adult worm begins passing gravid proglottids (Fig. 31.8). These segments disintegrate and release eggs (Fig. 31.9), which pass in feces to the external environment. Flea larvae ingest the eggs. As with *H. diminuta*, the oncosphere



Figure 31.7. Scolex of *Dipylidium caninum*. Note the four suckers and hooks.

Dipylidium caninum

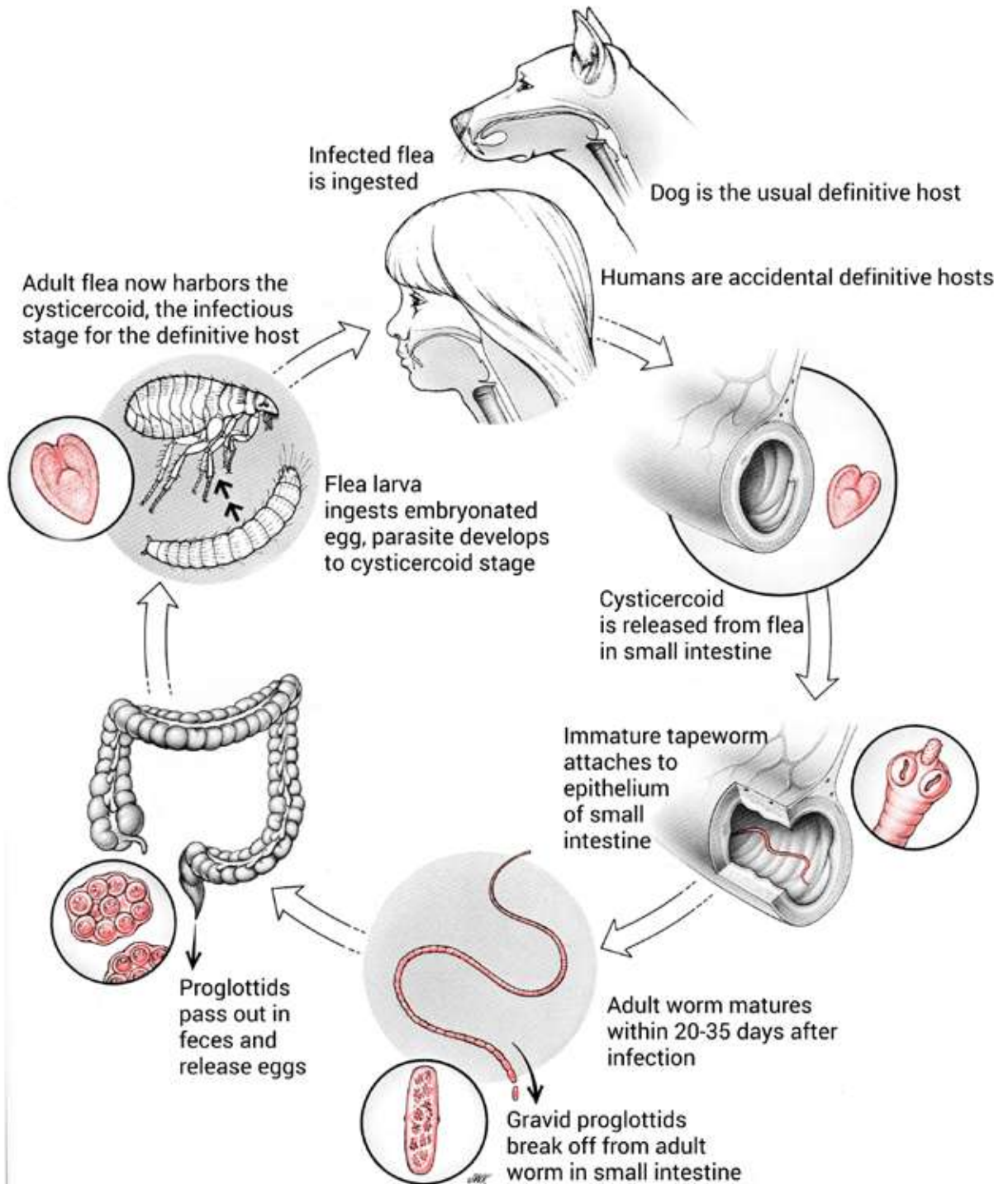




Figure 31.8. Double-pored gravid proglottid of *Dipylidium caninum*. 200 μ m in width.



Figure 31.9. Egg cluster of *Dipylidium caninum*.

penetrates the hemocele of the immature insect host and develops into the cysticercoid. This stage is infective for humans. Coming in close contact with dogs or cats and inadvertently swallowing an infected adult flea can infect children.

Clinical Disease

D. caninum does not usually cause any recognized clinical disease, but a few case reports have suggested several symptoms, such as mild abdominal pain, diarrhea, pruritus ani, failure to thrive, and irritability, which may be associated with infection.⁴²⁻⁴⁵

Most *D. caninum* infections occur in children less than 8 years of age.⁴⁶

Diagnosis (see Clinical Appendix)

The diagnosis is made by microscopically identifying the characteristic egg clusters (Fig. 31.9) in the patient's stool. If proglottids are available, they, too, are readily identifiable.

Treatment (see Clinical Appendix)

Praziquantel or niclosamide are the drugs of choice.^{47,48}

Prevention and Control

Eradication of fleas in pets and treating infected animals with niclosamide reduce the chances of human infection.

References

1. Gomez-Priego, A., et al., Detection of serum antibodies in human *Hymenolepis* infection by enzyme immunoassay. *Trans Trop Med Hyg* **1991**, 85, 645-647.
2. Crompton, D. W., How much human helminthiasis is there in the world? *J Parasitol* **1999**, 85 (3), 397-403.
3. Thompson, R. C., Neglected zoonotic helminths: *Hymenolepis nana*, *Echinococcus canadensis* and *Ancylostoma ceylanicum*. *Clin Microbiol Infect* **2015**, 21 (5), 426-32.
4. Mirdha, B. R.; Samantray, J. C., *Hymenolepis nana*: a common cause of paediatric diarrhoea in urban slum dwellers in India. *Journal of tropical pediatrics* **2002**, 48 (6), 331-4.
5. Sirivichayakul, C., et al., *Hymenolepis nana* infection in Thai children. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet* **2000**, 83 (9), 1035-8.
6. Heyneman, D., Studies on helminth immunity. III. Experimental verification of autoinfection from cysticercoids of *Hymenolepis nana* in the white mouse. *J Infect Dis* **1961**, 109, 10-8.
7. Ito, A., *Hymenolepis nana*: immunogenicity of a lumen phase of the direct cycle and failure of autoinfection in BALB/c mice. *Exp Parasitol* **1982**, 54 (1), 113-20.

8. Muehlenbachs, A., et al., Malignant Transformation of *Hymenolepis nana* in a Human Host. *N Engl J Med* **2015**, 373 (19), 1845-52.
9. Macnish, M. G., et al., Detection of the rodent tapeworm *Rodentolepis* (= *Hymenolepis*) *microstoma* in humans. A new zoonosis? *International journal for parasitology* **2003**, 33 (10), 1079-85.
10. Bilharz, T.; von Siebold, C. T., Ein Beitrag zur Helminthographia humana, aus brieflichen Mitteilungen des Dr. Bilharz in Cairo, nenst Bermerkungen von Prof. C. Th. von Siebold in Breslau. *Z. Wiss. Zool.* **1852**, 4, 53-76.
11. Grassi, B., Entwicklungscyclus der *Taenia nanna*. Dritte Preliminarnote. *Centralblatt für Bakteriologie und Parasitenkunde* **1887**, 2, 305-312.
12. Riley, W. A.; Shannin, W. R., The Rat Tapeworm, *Hymenolepos diminuta*. In Man. *The Journal of Parasitology* **1922**, 8 (3), 109-117.
13. Fan, P. C., Infectivity and development of the human strain of *Hymenolepis nana* in ICR mice. *Southeast Asian J Trop Med Public Health* **2005**, 36 (1), 97-102.
14. Andreassen, J., et al., Intestinal Tapeworms. London, Arnold,: 1998; p 520-537.
15. Menan, E. I., et al., [Profile of intestinal helminthiasis in school aged children in the city of Abidjan]. *Bull Soc Pathol Exot* **1997**, 90 (1), 51-4.
16. Ito, A., et al., *Hymenolepis nana*: immunity against oncosphere challenge in mice previously given viable or non-viable oncospheres of *H. nana*, *H. diminuta*, *H. microstoma* and *Taenia taeniaeformis*. *Int J Parasitol* **1991**, 21 (2), 241-5.
17. Palmas, C., et al., Transfer of immunity against *Hymenolepis nana* in mice with lymphoid cells or serum from infected donors. *Parasitology* **1984**, 89 (Pt 2), 287-93.
18. Niwa, A.; Miyazato, T., Reactive oxygen intermediates from eosinophils in mice infected with *Hymenolepis nana*. *Parasite immunology* **1996**, 18 (6), 285-95.
19. Conchedda, M., et al., Immune response to the cestode *Hymenolepis nana*: cytokine production during infection with eggs or cysts. *International journal for parasitology* **1997**, 27 (3), 321-7.
20. Asano, K.; Muramatsu, K., Importance of interferon-gamma in protective immunity against *Hymenolepis nana* cysticercoids derived from challenge infection with eggs in BALB/c mice. *International journal for parasitology* **1997**, 27 (11), 1437-43.
21. Watanabe, N., et al., Expulsion of *Hymenolepis nana* from mice with congenital deficiencies of IgE production or of mast cell development. *Parasite immunology* **1994**, 16 (3), 137-44.
22. Bouree, P., [Efficacy of a single dose of praziquantel as treatment for *Taenia saginata* and *Hymenolepis nana*]. *Pathol Biol (Paris)* **1988**, 36 (5 Pt 2), 759-61.
23. Bouree, P., Successful treatment of *Taenia saginata* and *Hymenolepis nana* by single oral dose of praziquantel. *J Egypt Soc Parasitol* **1991**, 21 (2), 303-7.
24. Pedro Rde, J., et al., [Treatment of school children with *Hymenolepis nana* with praziquantel]. *AMB Rev Assoc Med Bras* **1982**, 28 (9-10), 216-7.
25. Schenone, H., Praziquantel in the treatment of *Hymenolepis nana* infections in children. *Am J Trop Med Hyg* **1980**, 29 (2), 320-1.
26. Maggi, P., et al., *Hymenolepis nana* parasites in adopted children. *Clin Infect Dis* **2005**, 41 (4), 571-2.
27. Diaz, E., et al., Epidemiology and control of intestinal parasites with nitazoxanide in children in Mexico. *Am J Trop Med Hyg* **2003**, 68 (4), 384-5.
28. Rossignol, J. F.; Maisonneuve, H., Nitazoxanide in the treatment of *Taenia saginata* and *Hymenolepis nana* infections. *Am J Trop Med Hyg* **1984**, 33 (3), 511-2.
29. Chero, J. C., et al., *Hymenolepis nana* infection: symptoms and response to nitazoxanide in field conditions. *Trans R Soc Trop Med Hyg* **2007**, 101 (2), 203-5.
30. Rudolphi, K. A., Entozoorum Synopsis cui Accedunt Mantissa Duplex et Indices Locuplet. Berolini :Sumtibus A. Rücker: 1819.
31. Weinland, D. F., Human Cestodes. An Essay on the Tapeworms of Man. 1858.
32. Pappas, P. W.; Barley, A. J., Beetle-to-beetle transmission and dispersal of *Hymenolepis diminuta* (Cestoda) eggs via the feces of *Tenebrio molitor*. *J Parasitol* **1999**, 85 (2), 384-5.
33. Arai, H. P., Biology of the Tapeworm *Hymenolepis diminuta*. 1980.
34. Andreassen, J., et al., Immunology and biochemistry of *Hymenolepis diminuta*. *Advances in*

- parasitology* **1999**, 42, 223-75.
35. Kalaivani, R., et al., *Hymenolepis diminuta* infection in a school-going child: A rare case report. *Australas Med J* **2014**, 7 (9), 379-81.
 36. Kilincel, O., et al., [A rare case of *Hymenolepis diminuta* infection in a small child]. *Mikrobiyol Bul* **2015**, 49 (1), 135-8.
 37. Wiwanitkit, V., Overview of *Hymenolepis diminuta* infection among Thai patients. *MedGenMed : Medscape general medicine* **2004**, 6 (2), 7.
 38. Hamrick, H. J., et al., Rat tapeworm (*Hymenolepis diminuta*) infection in a child. *The Pediatric infectious disease journal* **1990**, 9 (3), 216-9.
 39. Tena, D., et al., Human infection with *Hymenolepis diminuta*: case report from Spain. *Journal of clinical microbiology* **1998**, 36 (8), 2375-6.
 40. Dwinell, M. B., et al., Tapeworm infection decreases intestinal transit and enteric aerobic bacterial populations. *The American journal of physiology* **1997**, 273 (2 Pt 1), G480-5.
 41. Ostlind, D. A., et al., The *Hymenolepis diminuta*-golden hamster (*Mesocricetus auratus*) model for the evaluation of gastrointestinal anticestode activity. *The Journal of parasitology* **2004**, 90 (4), 898-9.
 42. Hamrick, H. J., et al., Two cases of dipylidiasis (dog tapeworm infection) in children: update on an old problem. *Pediatrics* **1983**, 72 (1), 114-7.
 43. Manyam, K. S., et al., Observations in a case of *Dipylidium caninum* infection. *Vet Med Small Anim Clin* **1980**, 75 (1), 66.
 44. Narasimham, M. V., et al., *Dipylidium caninum* infection in a child: a rare case report. *Indian J Med Microbiol* **2013**, 31 (1), 82-4.
 45. Szwaja, B., et al., A case of *Dipylidium caninum* infection in a child from the southeastern Poland. *Wiad Parazytol* **2011**, 57 (3), 175-8.
 46. Molina, C. P., et al., Infection by *Dipylidium caninum* in an infant. *Archives of pathology & laboratory medicine* **2003**, 127 (3), e157-9.
 47. Jones, W. E., Niclosamide as a treatment for *Hymenolepis diminuta* and *Dipylidium caninum* infection in man. *Am J Trop Med Hyg* **1979**, 28 (2), 300-2.
 48. Schenone, H., et al., [Infection by *Dipylidium caninum* in a young girl treated with praziquantel]. *Bol Chil Parasitol* **1987**, 42 (3-4), 74-5.

32. Juvenile Tapeworm Infections of Humans

Echinococcus granulosus

(Batsch 1786)

Pronunciation: \i-,kī-nə-'kā-kəs\ \gran-yə-lō-sis\

Echinococcus multilocularis

(Leuckart 1863)

Pronunciation: \i-,kī-nə-'kā-kəs\ \mul-tī-lāk-yü-ler-is\

Introduction

Echinococcus granulosus (\eh-KYE-no-KOK-us\ \GRAN-you-low-sis\) a taenid tapeworm in the order Cyclophyllidea. It lives as an adult parasite in the small intestine of its definitive host – the domestic dog and other Canidae. It is one of the smallest cestodes, measuring 5 mm in length. The strobila consists of a scolex and three segments (Fig. 32.1). Sheep and other herbivores serve as intermediate hosts, acquiring infection by eating embryonated eggs that contaminate grazing pastures. Humans are also susceptible to the juvenile stage of the parasite, which may develop into a large, fluid-filled cyst, often exceeding 40 cm in diameter. The condition is referred to as hydatid disease. Although both *E. granulosus* and *E. multilocularis* (\eh-KYE-no-KOK-us\ \MUL-tee-lōk-you-lair-is\) cause infection in humans, 95% of the cases of human disease are due to *E. granulosus*.¹ The WHO estimates that over 1 million people suffer from hydatid disease.²

Distribution of *E. granulosus* coincides with sheep husbandry. Eurasia, Russia and Central Asia and China (including Tibet), Mediterranean countries (Turkey, Lebanon, and Syria), North and East Africa (Egypt, Sudan, and Kenya), and Australia have the highest prevalences.^{3,5} Mongolia is also an important source of hydatid disease.⁶ Infection has been

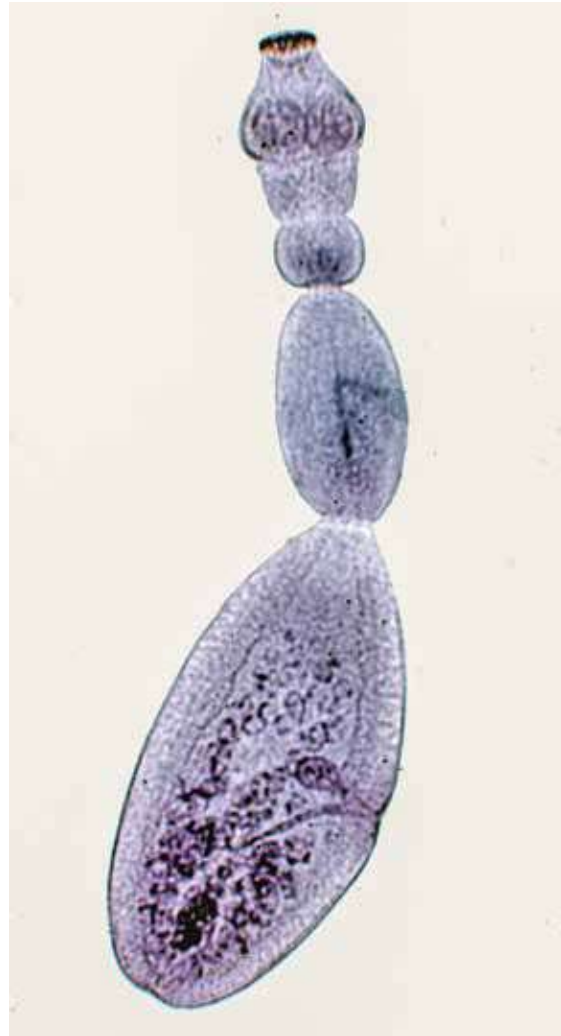


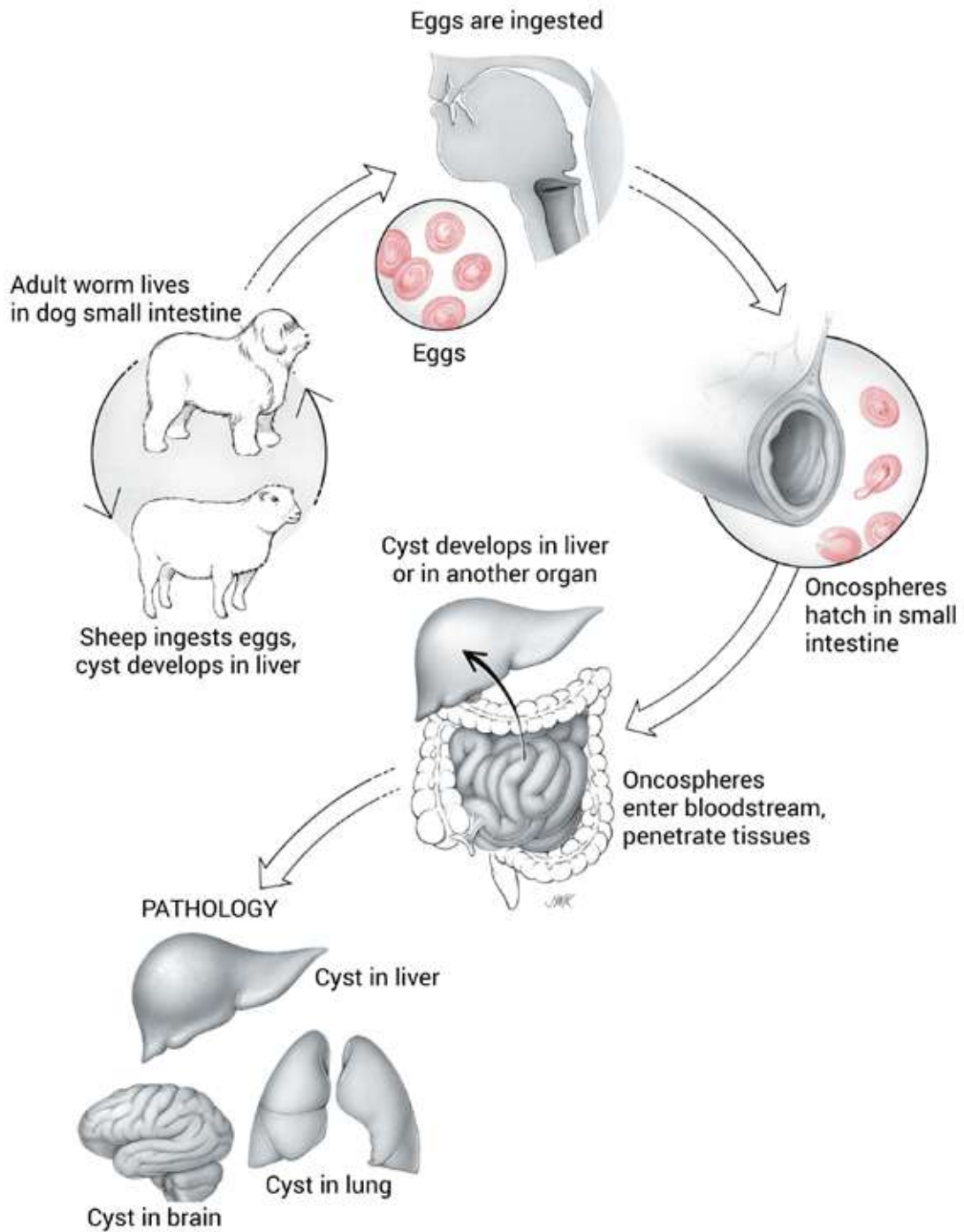
Figure 32.1. Adult of *Echinococcus granulosus*. Note suckers and hooks on scolex. The worm is 5.5 mm long.

eradicated from Iceland, Ireland, and Greenland but not from other island communities, such as Cyprus and New Zealand.^{2,7}

Small, endemic areas of infection exist in the United States, primarily in California, Utah, New Mexico, Alaska, and Arizona, where local transmission to humans has been documented.⁸⁻¹⁰ A number of cases have also been described in the lower Mississippi River Valley.¹¹

Indigenous peoples of the Canadian Arctic, especially the Inuit, are infected with a northern variant, *E. granulosus* var. *canadensis*,

Echinococcus granulosus



acquired in a sylvatic cycle involving moose and caribou (cervids), wolves, and sled dogs.³

^{12, 13} Two cases reported from Alaska were unusually severe.¹³ In northern areas in certain Scandinavian countries, herding of reindeer and the artificial introduction of reindeer is associated with this infection.¹⁴

Historical Information

Descriptions of the massive cysts seen in slaughtered animals can be found in multiple ancient texts from Babylon, Greece, and Rome.¹⁵ In 1766, Pierre Pallas described the hydatid cyst of *E. granulosus*, in which he sketched cysts that he removed from the viscera of mice and compared them with those recovered by others from human infections.¹⁶ In 1782, Johann Goeze, in his classic monograph, depicted the juveniles (i.e., protoscolices).¹⁷ In 1853, Carl von Siebold described the adult worms in the dog and demonstrated that echinococcus cysts from sheep gave rise to adult tapeworms when fed to dogs.¹⁸ In 1863, Bernhard Naunyn fed the contents of hydatid cysts from a human infection to dogs, and recovered adult tapeworms in them.¹⁹ These experiments provided the essential link between the animal and human infection cycles.

Life Cycle

E. granulosus adults live in the canine small intestine. Multiple infections are the rule, with hundreds to thousands of adult worms occupying the more significant portion of the upper half of the small intestine. The gravid segment (one per adult tapeworm) breaks off and disintegrates in the large bowel, releasing hundreds of infective eggs (Figs. 28.5, 29.5), which then pass out with the feces. Sheep, other domestic animals (e.g., cattle, pigs, and horses), and humans acquire the juvenile stage by ingesting embryonated eggs. Each

intermediate host species (sheep, cattle, pig, and horse) seems to have evolved a separate, genetically definable strain of parasite.²⁰

The oncosphere hatches in the small intestine, enters the bloodstream, and, in the vast majority of cases, reaches the liver via the portal circulation (Fig. 32.2).²¹⁻²³ Other organs can also be invaded, including the lung, brain, heart, bones, eyes, and kidney.²⁴⁻³⁰ Once in the tissue, the larva synthesizes a hyaline membrane and becomes surrounded by it. This membrane differentiates into an outer, acellular laminate structure and an inner, cellular germinal layer (Fig. 32.3, 32.11). The inner surface of the germinal layer gives rise to protoscolices (Fig. 32.5) (i.e., the infectious stage for the definitive host), and more outer membrane material.

The hydatid cyst requires several months to years in order to develop, mature and fill with fluid (Fig. 32.6). The fluid is under pressure, and the wall, while substantial, can rupture if severely traumatized. The entire cyst can contain millions of protoscolices. The diameter of the mature outer cyst varies from 2–20 cm, and sometimes is even larger. The fluid portion of the cyst contains both host and parasite proteins. To complete the life cycle, a canine host must ingest the hydatid cyst and its contents (i.e., the protoscolices). This commonly occurs when infected sheep are slaughtered (Fig. 32.11), and organs containing hydatid cysts are discarded or fed to dogs.

The protoscolex, released from the hydatid cyst, attaches itself to the wall of the small intestine aided by its four suckers and a row of hooklets (Fig. 32.6). New gravid proglottids are produced within about 2 months. Dogs do not seem to become ill from the effects of even heavy intestinal infections, which may exceed a million adult worms.

Cellular and Molecular Pathogenesis

There is minimal host reaction to a living hydatid cyst. Little is known regarding the nature of the immune responses directed at the parasite and the antigens it secretes into the cyst fluid. Evidence suggests that the production of immunosuppressive substances by the parasite suppresses host responses for the life of the cyst.³¹⁻³⁴ Studies indicate a potential role for interleukin-21 in echinococcosis.³⁵ Cysts can remain alive for months to years. In contrast to the cysts, the eggs and the oncospheres of *E. granulosus* are immunogenic and elicit protective immunity. This feature might provide a mechanism by which intermediate hosts, including humans, control the number of oncospheres that ultimately develop into hydatid cysts.³

Clinical Disease

The primary exposure and initial infection are thought always to be asymptomatic, with

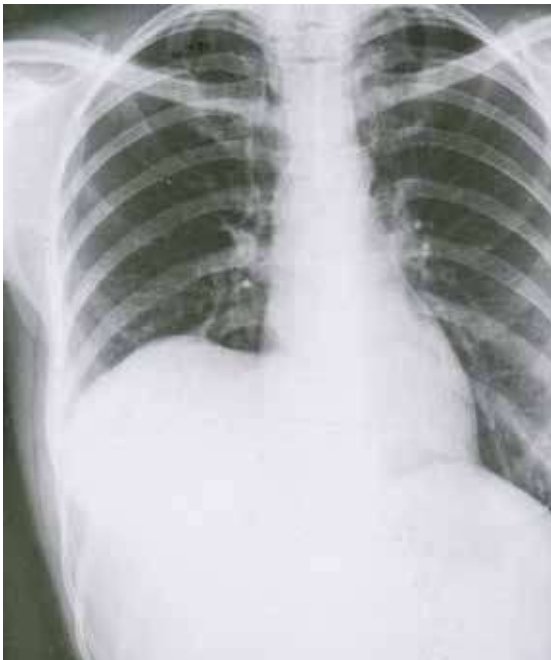


Figure 32.2. Radiogram of upper body showing elevation in right lobe of liver due to a large hydatid cyst.



Figure 32.3. Histological section of an hydatid cyst with capsules filled with protoscolices (arrows) of *Echinococcus granulosus*. Each protoscolex measures approx. 13-14 μm in length.

symptoms only occurring coincident with cyst development. Hydatid disease develops due to rapid growth of the cyst (or cysts), and subsequent expansion of the cyst wall. The liver is the most common site of hydatid cyst development (Fig. 32.9), followed by the lungs. More than 90% of cysts develop in these two organs. Cysts have been reported to infect almost all of the visceral organs, including the bone marrow (Fig. 32.10) or brain, the latter usually with fatal results.

If a cyst ruptures, the entire contents (fluid and protoscolices) spill into the surrounding tissues or body cavity (e.g., peritoneum, pleural cavity). When such an event occurs, an immediate anaphylactic reaction may ensue.^{36, 37} Moreover, cyst contents can seed the area, and invade new tissues to produce second-generation hydatid cysts (Fig. 32.10). Even a few cells from the germinal membrane can re-establish an entire hydatid cyst, as each cell of the germinal membrane has a stem cell-like ability to reproduce a



Figure 32.4. Brood capsule with protoscolices of *Echinococcus granulosus*.

full hydatid cyst. Avoiding host contact with hydatid cyst contents and sterilizing the germinal layer is essential as the mortality rate of 2–4%, usually seen in cases of cystic echinococcus, may be increased if patients are improperly treated.

Approximately 60% of hydatid cysts are asymptomatic. Symptoms, when present, are those of a large space-occupying lesion. If the liver is involved, it becomes enlarged (Fig. 32.3). The cyst presents as a palpable, soft, non-tender, intrahepatic mass. The uninvolved bulk of the liver remains normal. Involvement of the lung is usually identified by chance on a radiograph, or by the presence of bloody or ‘salty’ sputum, in which protoscolices or hooklets (Fig. 32.7) can be found. Patients may describe the sputum they produce as salty if it is due to a leaking hydatid cyst in the lungs.³⁸ Expansion and rupture of liver hydatid cysts into the biliary tree can result in secondary cholangitis, biliary obstruction, and intraperitoneal rupture.³⁹ Lung cysts can rupture into the bronchial tree and cause the development of a bronchopulmonary fistula. Rupture of a cyst, wherever it is lodged, may occur even after relatively minor blunt trauma, and may lead to an allergic reaction. It may be mild and limited to

urticaria, or may take the form of anaphylactic shock, requiring immediate intervention. Most patients with the northern variant of echinococcosis have asymptomatic lung cysts that are usually detected on chest radiographs obtained for other reasons (e.g., tuberculosis screening).

Diagnosis (see Clinical Appendix)

An accurate case history is essential to the diagnosis of hydatid disease. Ownership of dogs, life on a sheep farm – even during childhood, and especially in endemic areas – and/or a history of travel to endemic areas are important factors for this disease.

The diagnostic algorithms for hydatid disease have been modified based on increasing experience with radiologic imaging modalities, including ultrasound, CT, and MRI. Currently ultrasound is the recommended modality with a reported 90% sensitivity for hepatic hydatidosis but this is still controversial as some investigations suggest CT might be superior.⁴⁰ Despite this recommendation, in many resource-rich countries both CT and



Figure 32.5. Protoscolex of *E. granulosus*.



Figure 32.6. Petri dish filled with daughter cysts of *Echinococcus granulosus*.

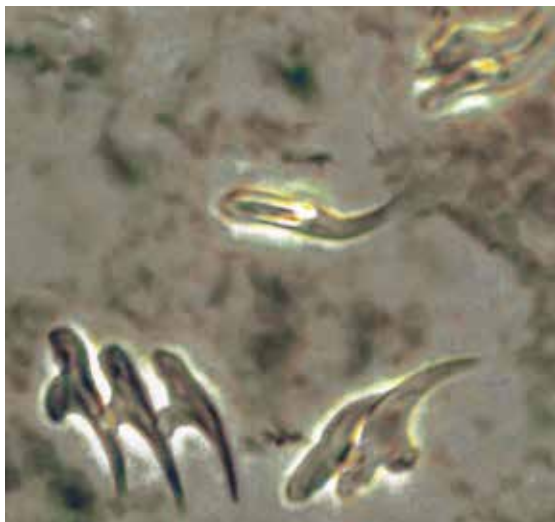


Figure 32.7. Hooklets of a protoscolex. Each hooklet measures approx. 2 μm in length.

MRI are widely used.¹ The major radiological criteria have been standardized and management is now guided by an expert consensus generated under the aegis of the World Health Organization's Informal Working Group on Echinococcosis.⁴¹ Imaging frequently reveals a mixture of hyperdensities and hypodensities, with scattered calcifications (Fig. 32.8).⁴² Internal septae and daughter cysts are often demonstrable, and such evidence is strongly suggestive of the diagnosis.

Serodiagnosis is also useful both for primary diagnosis and following patients during and after their medical or surgical management.⁴³ ELISA, for the detection of echinococcus antibodies, is the test of choice, and employs hydatid fluid antigen. In addition, efforts are underway to refine the ELISA using specific antigens, including two known as antigen 5 and lipoprotein antigen B.⁴⁴ Of patients with hepatic cystic echinococcosis, 30–40% have negative serologies, possibly due to the ability of *E. granulosus* antigens to inhibit B cell activity and proliferation.⁴⁵ Patients with intact cysts have a high false-negative rate; presumably because these patients do not experience sufficient antigen challenge to induce a detectable antibody response. Efforts are underway to develop tests that detect circulating echinococcus antigens.⁴⁶ Eosinophilia is an uncommon finding in patients with hydatid disease.⁴⁷

A definitive diagnosis of echinococcosis



Figure 32.8. Radiogram of a calcified hydatid cyst of *Echinococcus granulosus*.

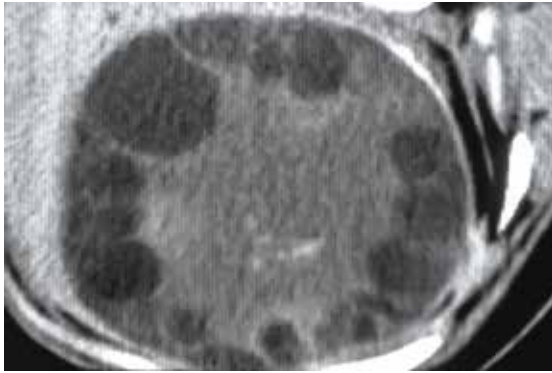


Figure 32.9. Radiogram of a liver infected with multiple hydatid cysts of *Echinococcus granulosus*.

can be made by microscopically identifying hooklets (Fig. 32.7) in any sample, sputum being the most common. This is usually the case in long-term infections, in which all cells within the cyst have died, and the only remaining evidence of infection is the hooklets and portions of the acellular, laminate outer membrane of the cyst wall. Biopsy of



Figure 32.10. Femur from a patient who died of hydatid disease of the bone. Courtesy W. Johnson.



Figure 32.11. Navajo woman butchering a sheep. The organs containing hydatid cysts are occasionally fed to their dogs, thus completing the life cycle. Courtesy P.M.Schantz.

cysts is contra-indicated due to their metastatic nature.

Treatment (see Clinical Appendix)

Treatment of hydatid disease is driven by cyst size, location, and stage.⁴⁸ Hydatid cysts undergo an increase in size of 1–5 cm per year, with calcification occurring 5–10 years post-infection, but this development varies depending on host and parasite factors.^{49, 50} Hydatid cysts develop, enlarge, fill with protoscolices and daughter cysts.⁴⁸ As the hydatid cyst develops, it goes through a series of defined stages that guide the choice of optimum therapy from cystic echinococcosis (CE) stages 1–5.

Treatment of patients with hydatid cysts often requires both surgical and medical interventions.⁴¹ Surgical intervention is the usual



Figure 32.12. Peruvian slaughter house. Organs from sheep containing hydatid cysts are routinely fed to the numerous dogs that frequent these unsanitary, unsupervised rural establishments.

approach to cure for patients with large cysts and complex cysts. Care must be taken to prevent inadvertent rupture of the cysts. Successful removal depends on the location of the cyst. Historically, various strategies have been devised to prevent or minimize spillage of cyst contents. This includes the preoperative use of antihelminthic drugs and protoscolicidal compounds such as 95% ethanol, hypertonic saline and cetrimide.

Introduced in the mid-1980s, the PAIR technique (Puncture, Aspiration, Injection, Reaspiration) with ultrasound guidance has replaced the need for laparotomy and surgery for some patients.^{51, 52} The risk of complications from PAIR appears to be significantly reduced through adjuvant antihelminthic chemotherapy started one month before performing this procedure. More recently, laparoscopic surgery has emerged as a viable alternative for surgical treatment.⁵³ For pulmonary cysts antihelminthic therapy is usu-

ally started after cyst removal with concerns that preoperative antihelminthic treatment may weaken the cyst wall, leading to cyst rupture.

Albendazole 400 mg BID x 1–6 months or, for children, 15 mg/kg/d (max. 800 mg) x 1–6 months has been used successfully to treat hydatid disease, particularly when surgical removal was impossible. Chemotherapy can result in cyst regression or collapse, although prolonged therapy courses are usually required.^{47, 51, 52} It has been estimated that treatment can result in the disappearance of up to 48% of cysts and a substantial reduction in size of an additional 24%.⁵² Albendazole is preferable to mebendazole because the former is metabolized to a sulfoxide derivative, exhibiting antiparasitic activity and is widely distributed in the tissues. In many areas of the world, praziquantel is added, and patients are given both albendazole and praziquantel in response to evidence of praziquantel's protoscolicidal activity.^{54–57} Therapeutic responses can be monitored radiographically and serologically. Reversible liver toxicity has been reported with prolonged therapy with albendazole.^{58, 59} As outlined in the section on trichuriasis, benzimidazole antihelminthics, including albendazole, are teratogenic and embryotoxic in laboratory animals.



Figure 32.13. *Echinococcus multilocularis* alveolar cyst from an infected vole.

Both drugs are considered category C agents in the United States. Before use in pregnancy, the risks versus benefits must be weighed.

Prevention and Control

Infection of domestic dogs with *E. granulosus* can be prevented. Control is best achieved by avoiding feeding dogs (Figs. 32.11, 32.12) any infected organs of slaughtered sheep or other animals and by periodically treating dogs prophylactically with niclosamide, arecoline hydrobromide, or praziquantel.⁵² An arecoline control program in dogs has resulted in the near-elimination of *E. granulosus* infection in New Zealand and Tasmania. In Iceland, the mass slaughter of infected sheep and dogs led to the total eradication of the disease. Attempts to duplicate that effort in Cyprus were not successful.⁶⁰

Strict regulations regarding the importation of animal products that might carry *E. granulosus* eggs (e.g., animal hides of various carnivores, fishing flies, etc.) is a requirement for this control strategy to be effective. A recombinant peptide vaccine, EG95, induces high levels of protection in sheep and should prove helpful in certain parts of the world.^{61, 62} Canine vaccination, which is currently being tested, may be critical in many areas of the world.^{63, 64}

Echinococcus multilocularis

Pronunciation: \i-, kī-nə-'kā-kəs\\mul-tī-lāk-yū-ler-is\

Echinococcus multilocularis (\eh-KYE-no-KOK-us\\MUL-tee-lok-you-lair-is\)) infects wild Caenidae, such as fox, and can infect domestic dogs. The intermediate hosts are rodents (e.g., voles, field mice, ground squirrels).⁶⁵ The biology of the human infection resembles the situation found in the inter-

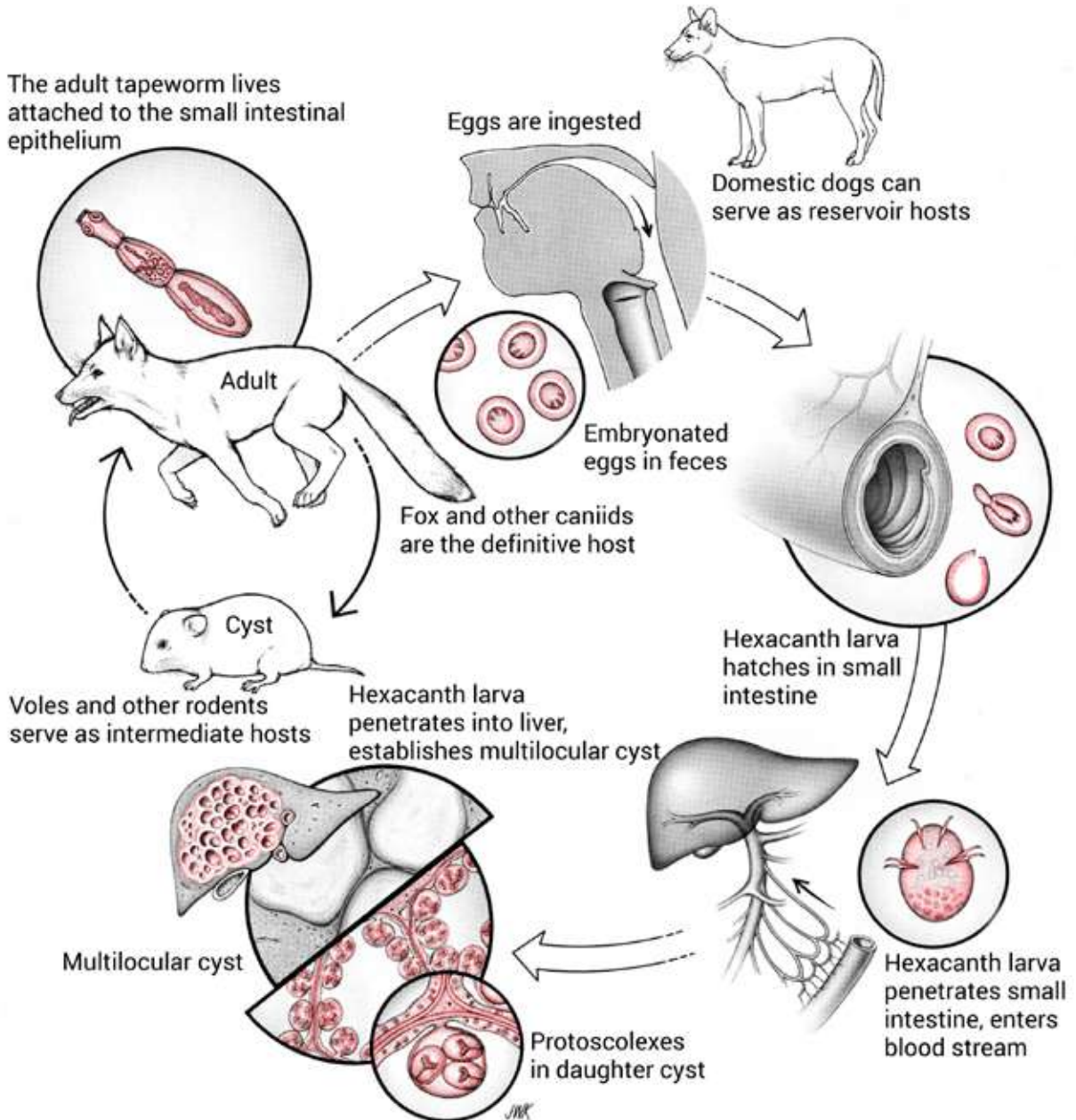
mediate hosts. *E. multilocularis* results in discrete cysts (alveolar echinococcosis) (Fig. 32.13) that metastasize from a single location, except for the fact that *E. multilocularis* does not produce protoscolices in human infection, only membranes that grow and bisect whatever organ they happen to be in, confounding the diagnosis of this unusual parasitic disease. In rodents, protoscolices are found in each daughter cyst.

This infection is prevalent among fur trappers and others whose occupations bring them in close contact with wild foxes and populations with close contact with dogs that regularly feed on infected rodents,⁶⁶ and in urban areas where reservoir hosts can expand from foxes to domesticated dogs and cats. Alveolar echinococcosis has emerged to the point where it is endemic in the northern hemisphere, including western Europe, where it is considered a re-emerging disease due, in part, to the banning of fox hunting in several Common Market countries.⁶⁷ It is also prevalent in the Russia, Central Asia, China (especially in western and central regions), and northern Japan (Hokkaido). In North America, human infections have been reported from Alaska and in the upper Midwest and Northern Plains along the Canadian border.

Life Cycle

Infection begins when the intermediate host ingests the egg. The oncosphere hatches in the small intestine and invades the liver by the hematogenous route. It transforms and then grows into an alveolar type of cyst, characterized by numerous daughter cysts, as compared to hydatid cysts, which grow as a single membrane-bounded unit. Foxes and dogs ingest infected livers containing numerous protoscolices, which leads to infection with the adult worm, thereby completing the life cycle.

Echinococcus multilocularis



Clinical Disease

In humans, the incubation period of larval infection with *E. multilocularis* is long, with incubation periods of 5–15 years. The initial exposure often occurs during childhood, but the disease has been recognized mainly in older adults.⁶⁸ Alveolar disease can be highly aggressive, affecting the liver as the primary target organ. The membrane proliferates indefinitely and causes progressive destruction of the liver parenchyma, which can lead to hepatic failure. In some cases, this may lead to a direct extension to the lungs and rarely metastasis to the CNS.⁶⁹ Although initially asymptomatic, as the disease progresses, infected individuals may develop hepatomegaly, jaundice, multiple palpable abdominal masses, epigastric pain, and weight loss. If untreated, the mortality of clinically apparent disease can be as high as 50–75%.⁶⁶

HLA type may affect an individual's risk of further disease after chemotherapy. Regrowth of the parasite was significantly more prevalent in patients with haplotype HLA-DQB1*02 than those with haplotype HLA-DRB1*1157.⁷⁰ Interleukin-10 production was also higher in infected individuals and may relate to the lack of development of protective immunity.⁷¹ Other investigators have noted an important role of interleukin-5 and a robust Th2 response to infection with *E. multilocularis*.⁷²

Diagnosis (see Clinical Appendix)

Diagnosis is based on radiographic imaging findings showing tumor-like lesions with calcification areas.⁷³ Serologic testing by ELISA is highly sensitive and specific, allowing distinction between *E. multilocularis* and *E. granulosus*.^{74, 75} Directed biopsy of the lesion can often establish the diagnosis.⁶⁵

Treatment (see Clinical Appendix)

Surgical resection is the preferred approach to treatment with the entire larval mass being removed as part of a hepatic wedge or pulmonary lobe resection.⁶⁶ Long-term adjuvant chemotherapy with albendazole or mebendazole improves the 10-year survival rate.⁷⁶ ⁷⁷ Nitazoxanide has demonstrated *in vitro* activity against *E. multilocularis* and may be a useful alternative therapy.⁷⁸

Prevention relates to handling animal furs, subsequent oral contamination with eggs, and transmission from infected dogs or cats to humans. Trappers and those involved in animal husbandry in the fur industry should be educated to exercise caution when handling carcasses and processing furs. The use of oral baits laced with therapeutic doses of praziquantel for the control of *E. multilocularis* infections in foxes has been effective in certain areas.⁷⁹ Monthly treatment of dogs at high risk with praziquantel may be an effective approach.

Mesocestoides spp.

(Valliant 1863)

Pronunciation: \me-zə-ses-'tòi-dēz\

Introduction

Cestodes, in the genus *Mesocestoides* (\MEH-zo-SES-toy-DEEZ\), infect numerous species of mammals and birds. Human infections have been reported.⁸⁰ Most cases reported from the U.S. were caused by *Mesocestoides variabilis*.⁸¹ The life cycle of *Mesocestoides* is complex, involving a coprophagous arthropod as the first intermediate host and birds, snakes, lizards, amphibians, rodents, or other mammalian carnivores as the second intermediate host. The infective

stage, known as a tetrathyridium, develops in the second intermediate host. Tetrathyridia are usually about 1 cm long and contain an invaginated scolex with four suckers.

Humans acquire the parasite by eating the tetrathyridia, which can develop into an adult worm in the gut or migrate to the peritoneal cavity. Cases of *Mesocostoides* infection have been described in the U.S., but the mode of acquisition is not known.⁸⁰ A case of *Mesocostoides* infection from a 19-month-old boy in Alexandria, Louisiana, has led to the suggestion that the infection is food-borne, possibly in association with the culinary customs of the Acadian and Creole communities in this region. Treatment of the adult tapeworm with praziquantel or niclosamide is effective.⁸¹

***Spirometra* spp.** (Mueller 1935)

Pronunciation: \spī- 'rām-ə-trə\

Spirometra mansonoides (\SPEE-rom-u-tru\) and other related *Spirometra* spp. are pseudophyllidian tapeworms that cause a closely related series of metacestode infections in humans (some are life-threatening), collectively referred to as sparganosis. Cases of sparganosis have been reported from Southeast Asia, Japan, China, especially on the island of Hainan, Africa, Italy, and the United States.^{82, 83} The definitive hosts for the many species of *Spirometra* are cats, birds, canines, and a number of other carnivores.^{82, 84}

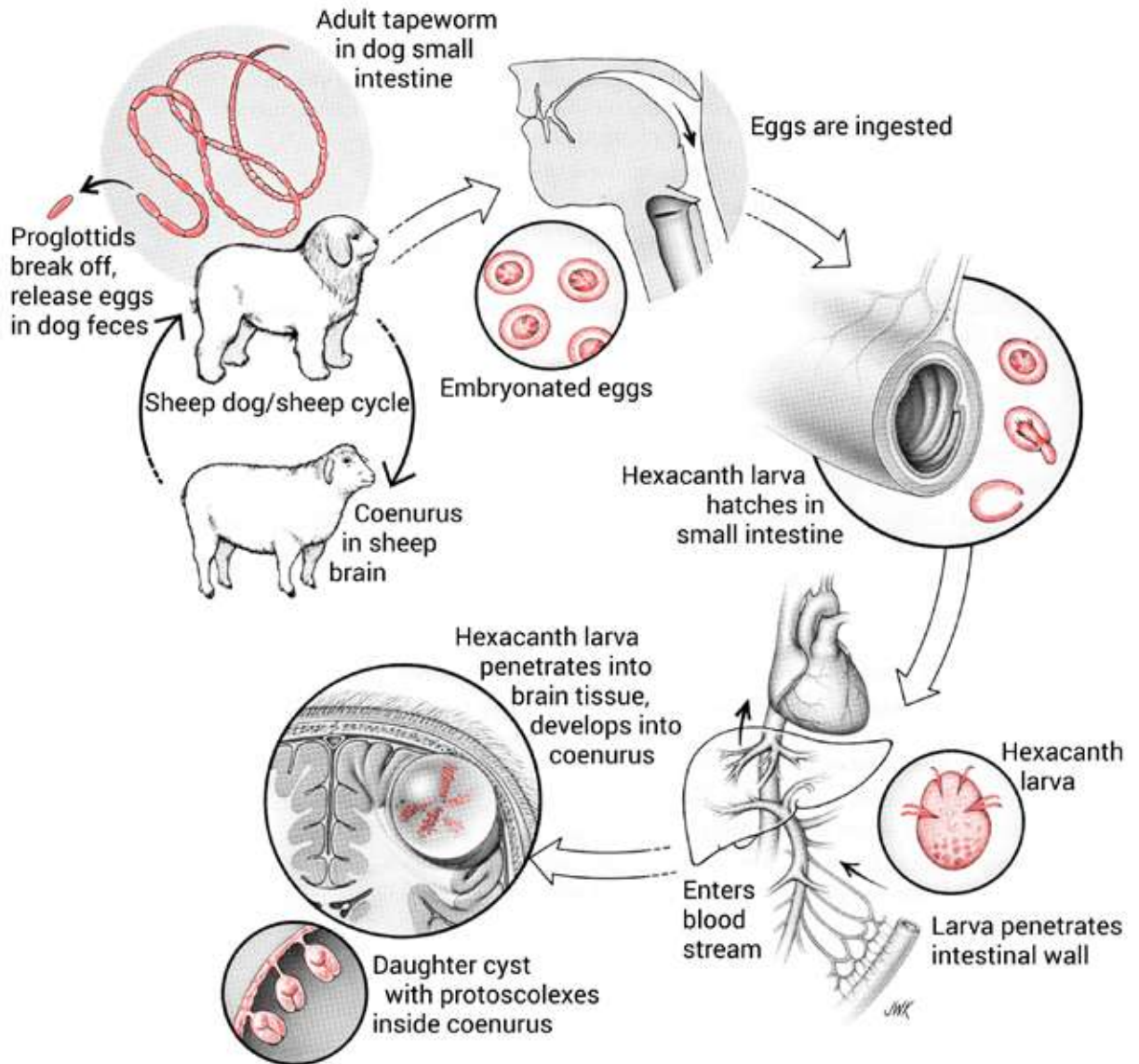
The life cycles of *Spirometra* spp. are complex and similar to that of *Diphyllbothrium latum* (see Chapter 30). The definitive hosts harbor the adult tapeworm in their small intestinal tract. When they defecate into freshwater, the unembryonated eggs hatch and the released free-swimming coracidia are eaten by copepods. The parasites then develop into

the procercoid stage that is infectious for second intermediate hosts: amphibians, fish, reptiles, and birds. These animals ingest the infected copepods, releasing the procercoids that penetrate the intestinal tract and develop to the plerocercoid stage in muscle tissue. This stage is infective for definitive hosts; warm-blooded predator species that prey on these infected cold-blooded animals.⁸⁵

Human infection results from ingestion of raw or undercooked flesh of any of the numerous intermediate hosts, and from application of such flesh as poultices. This practice is very common in some areas of the world, particularly on the island of Hainan, where over 30% of the frogs harbor the juvenile stage of *Spirometra*.^{86, 87} The skin of numerous cold-blooded vertebrates contains a variety of closely related peptides, referred to as megainins.⁸⁸ These amphipathic peptides are related to gramicidin, and have potent antimicrobial activities.⁸⁹ Employing frog and fish skins for medical use has a chemotherapeutic basis. As with any other therapy, using cold-blooded vertebrate skin as a poultice can have unwanted “side effects.” In this case, the user may develop a parasitic infection.

The plerocercoid stage can migrate out of the poultice and into the subcutaneous tissues, stimulated by the rise in temperature from the human host. The immature parasite may enter the site if the poultice is placed over an open wound or the eye. In the eye, the inner surface of the lid is the initial site of infection.^{90, 91} The surrounding tissues proliferate, becoming edematous and painful (unilateral peri-orbital edema), because the larva secretes a version of growth hormone similar to its mammalian host.⁹² The pleurocercoid may migrate beyond the eyelid into the brain, where it continues to grow. Neurological symptoms follow.⁹³ Occasionally, patients die of neurological complications of infection with *Spirometra* spp..

Taenia multiceps



Diagnosis can be made based on identification of the worm after removal or biopsy. There is an ELISA test for sparganosis with high levels of sensitivity and specificity that can be useful in making the diagnosis.^{94,95} In the case of early eye involvement, removal of the larva from the space between the lower lid and the eyeball results in a cure. Larvae often migrate behind the eye and even into the brain via the optic nerve, making easy removal of the parasite difficult if not impossible.⁹⁶ In these instances, surgery is necessary. Subcutaneous lesions are often removed by surgery, as treatment with antihelminthic therapy, such as with praziquantel is associated with limited success.⁹⁶ Prevention is complex, because of the effectiveness and popularity of poultice use for a variety of medical problems, including photophobia due to chickenpox.⁹⁷

***Taenia* spp. (other than *T. saginata* and *T. solium*)**

Pronunciation: \tē-nē-ə\

Taenia (TEE-nee-ah) spp. (e.g., *T. multiceps*, *T. brauni*, *T. serialis*) are a group of cyclo-

phyllidian tapeworms that, as adults, infect dogs and other Canidae. Intermediate hosts include domestic cattle, horses, goats, and some wild herbivorous animals of Africa. Humans become infected with the metacystode (juvenile stage), known as coenurus, which resembles cysts in intermediate hosts. Human infections have been confined mainly to Africa, but a few cases have been described in France, England, and North and South America.

The larva may invade the CNS (i.e., brain, eyes, spinal cord). Other space-occupying lesions, such as those caused by cysticerci and hydatid cysts, resemble this infection.

Diagnosis is based on clinical, epidemiologic, and laboratory findings. No serologic tests are currently available.

Treatment involves surgery if the lesion is accessible. Although albendazole, praziquantel, and other antihelminthic therapies have been used, it is still being determined how efficacious these are.⁹⁸ Recommendations for prevention are limited because reservoir hosts include many species of wild animals.

References

1. Griffin, D. O., et al., Management of serology negative human hepatic hydatidosis (caused by *Echinococcus granulosus*) in a young woman from Bangladesh in a resource-rich setting: A case report. *IDCases* **2014**, 1 (2), 17-21.
2. Budke, C. M., et al., Global socioeconomic impact of cystic echinococcosis. *Emerg Infect Dis* **2006**, 12 (2), 296-303.
3. Garcia, H. H., et al., The Cysticercosis Working Group in Peru. *Lancet* **2003**, 361, 547-56.
4. Moro, P. L.; Schantz, P. M., Echinococcosis: historical landmarks and progress in research and control. *Ann Trop Med Parasitol* **2006**, 100 (8), 703-14.
5. Abdybekova, A., et al., Epidemiology of echinococcosis in Kazakhstan: an update. *J Helminthol* **2015**, 89 (6), 647-50.
6. Ito, A.; Budke, C. M., The present situation of echinococcoses in Mongolia. *J Helminthol* **2015**, 89 (6), 680-8.
7. Economides, P., et al., Control of *Echinococcus granulosus* in Cyprus and comparison with other island models. *Veterinary parasitology* **1998**, 79 (2), 151-63.
8. Stojkovic, M., et al., Diagnosing and staging of cystic echinococcosis: how do CT and MRI perform in comparison to ultrasound? *PLoS Negl Trop Dis* **2012**, 6 (10), e1880.

9. Moro, P.; Schantz, P. M., Cystic echinococcosis in the Americas. *Parasitology international* **2006**, *55 Suppl*, S181-6.
10. Schantz, P. M., Echinococcosis in American Indians living in Arizona and New Mexico: a review of recent studies. *American journal of epidemiology* **1977**, *106* (5), 370-9.
11. Daly, J. J., et al., Unilocular hydatid cyst disease in the mid-South. *JAMA* **1984**, *251* (7), 932-3.
12. Finlay, J. C.; Speert, D. P., Sylvatic hydatid disease in children: case reports and review of endemic *Echinococcus granulosus* infection in Canada and Alaska. *The Pediatric infectious disease journal* **1992**, *11* (4), 322-6.
13. McManus, D. P., et al., Short report: molecular genetic characterization of an unusually severe case of hydatid disease in Alaska caused by the cervid strain of *Echinococcus granulosus*. *Am J Trop Med Hyg* **2002**, *67* (3), 296-8.
14. Sweatman, G. K., The Significance of the Artificial Introduction of Reindeer (*Rangifer Tarandus*) and Moose (*Alces Alces*) in the Spread of Hydatid Disease (*Echinococcus Granulosus*). *Ann Trop Med Parasitol* **1964**, *58*, 307-14.
15. Cox, F. E., History of human parasitology. *Clin Microbiol Rev* **2002**, *15* (4), 595-612.
16. Pallas, P. S., Miscellanea Zoologica: Quibus Novae Imprimis Atque Obscure Animalium Species Describuntur Et Observationibus Iconibusque Illustrantur. 1766.
17. Goeze, J. A. E.; Blankenburg, P. A., Versuch einer Naturgeschichte der Eingeweiderwumer Thierischer Korper. *Pape* **1782**.
18. Seibold, C. T., Von Uber die Verwandlung der *Echinococcus*-brut in *Taenia*. 1853.
19. Naunyn, B., Uber die zu *Echinococcus hominis* gehorige *Taenia*. *Arch Anat Physiol Wissenschr Med* **1863**, 412-416.
20. Haag, K. L., et al., Breeding systems in *Echinococcus granulosus* (Cestoda; Taeniidae): selfing or outcrossing? *Parasitology* **1999**, *118* (Pt 1), 63-71.
21. Heath, D. D., et al., *Echinococcus granulosus*: the mechanism of oncosphere lysis by sheep complement and antibody. *Int J Parasitol* **1994**, *24* (7), 929-35.
22. Holcman, B., et al., Ultrastructure of oncosphere and early stages of metacestode development of *Echinococcus granulosus*. *Int J Parasitol* **1994**, *24* (5), 623-35.
23. Harris, A., et al., *Echinococcus granulosus*: ultrastructure of epithelial changes during the first 8 days of metacestode development *in vitro*. *Int J Parasitol* **1989**, *19* (6), 621-9.
24. Diaz-Menendez, M., et al., Management and outcome of cardiac and endovascular cystic echinococcosis. *PLoS Negl Trop Dis* **2012**, *6* (1), e1437.
25. Nourbakhsh, A., et al., Hydatid disease of the central nervous system: a review of literature with an emphasis on Latin American countries. *Neurol Res* **2010**, *32* (3), 245-51.
26. Rexiati, M., et al., Diagnosis and surgical treatment of renal hydatid disease: a retrospective analysis of 30 cases. *PloS one* **2014**, *9* (5), e96602.
27. Sen, S., et al., Primary intraocular hydatid cyst with glaucoma. *J Pediatr Ophthalmol Strabismus* **2003**, *40* (5), 312-3.
28. Sinav, S., et al., A primary intraocular hydatid cyst. *Acta Ophthalmol (Copenh)* **1991**, *69* (6), 802-4.
29. Babitha, F., et al., Hydatid cyst of bone. *Indian J Med Microbiol* **2015**, *33* (3), 442-4.
30. Pazarci, O., et al., Treatment of Bifocal Cyst Hydatid Involvement in Right Femur with Teicoplanin Added Bone Cement and Albendazole. *Case Rep Orthop* **2015**, *2015*, 824824.
31. Dixon, J. B., Echinococcosis. *Comparative immunology, microbiology and infectious diseases* **1997**, *20* (1), 87-94.
32. Gottstein, B.; Hemphill, A., Immunopathology of echinococcosis. *Chemical immunology* **1997**, *66*, 177-208.
33. Wang, Y., et al., Impairment of dendritic cell function and induction of CD4(+)CD25(+)Foxp3(+) T cells by excretory-secretory products: a potential mechanism of immune evasion adopted by *Echinococcus granulosus*. *BMC Immunol* **2015**, *16*, 44.
34. Zheng, Y., Strategies of *Echinococcus* species responses to immune attacks: implications for therapeutic tool development. *Int Immunopharmacol* **2013**, *17* (3), 495-501.
35. Zhang, F., et al., CCR7(lo)PD-1(hi) CXCR5(+) CD4(+) T cells are positively correlated with levels of IL-21 in active and transitional cystic echinococcosis patients. *BMC Infect Dis* **2015**, *15*, 457.

36. Nunnari, G., et al., Hepatic echinococcosis: clinical and therapeutic aspects. *World J Gastroenterol* **2012**, *18* (13), 1448-58.
37. Minciullo, P. L., et al., Anaphylaxis caused by helminths: review of the literature. *Eur Rev Med Pharmacol Sci* **2012**, *16* (11), 1513-8.
38. Nakamura, K., et al., A case of pulmonary and hepatic cystic Echinococcosis of CE1 stage in a healthy Japanese female that was suspected to have been acquired during her stay in the United Kingdom. *Am J Trop Med Hyg* **2011**, *85* (3), 456-9.
39. Irkoruucu, O., et al., The detection of cysto-biliary communications during surgery for liver hydatid cysts: let's speak the unspoken. *Clinics (Sao Paulo)* **2012**, *67* (2), 179.
40. Dhar, P., et al., Current trends in the diagnosis and management of cystic hydatid disease of the liver. *J Commun Dis* **1996**, *28* (4), 221-30.
41. Brunetti, E., et al., Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop* **2010**, *114* (1), 1-16.
42. Agildere, A. M., et al., MRI of hydatid disease of the liver: a variety of sequences. *Assist Tomogr* **1998**, *22*, 718-24.
43. Gottstein, B., Molecular and immunological diagnosis of echinococcosis. *Clinical microbiology reviews* **1992**, *5* (3), 248-61.
44. Chemale, G., et al., *Echinococcus granulosus* antigen B hydrophobic ligand binding properties. *Biochimica et biophysica acta* **2005**, *1747* (2), 189-94.
45. Zhang, W.; McManus, D. P., Recent advances in the immunology and diagnosis of echinococcosis. *FEMS immunology and medical microbiology* **2006**, *47* (1), 24-41.
46. Pagnozzi, D., et al., Diagnostic Accuracy of Antigen 5-Based ELISAs for Human Cystic Echinococcosis. *PLoS Negl Trop Dis* **2016**, *10* (3), e0004585.
47. Schaefer, J. W.; Khan, M. Y., Echinococcosis (Hydatid disease): lessons from experience with 59 patients. *Reviews of infectious diseases* **1991**, *13* (2), 243-7.
48. Gharbi, H. A., et al., Ultrasound examination of the hydatid liver. *Radiology* **1981**, *139* (2), 459-63.
49. Moro, P. L., et al., Human hydatidosis in the central Andes of Peru: evolution of the disease over 3 years. *Clin Infect Dis* **1999**, *29* (4), 807-12.
50. Frider, B., et al., Long-term outcome of asymptomatic liver hydatidosis. *J Hepatol* **1999**, *30* (2), 228-31.
51. Filice, C.; Brunetti, E., Use of PAIR in human cystic echinococcosis. *Acta tropica* **1997**, *64* (1-2), 95-107.
52. Pelaez, V.; Kugler, C., PAIR as percutaneous treatment of hydatid liver cysts. *Acta Tropica* **2000**, *75*, 197-202.
53. Chen, X., et al., The Comparison of 2 New Promising Weapons for the Treatment of Hydatid Cyst Disease: PAIR and Laparoscopic Therapy. *Surg Laparosc Endosc Percutan Tech* **2015**, *25* (4), 358-62.
54. Alvela-Suarez, L., et al., Safety of the combined use of praziquantel and albendazole in the treatment of human hydatid disease. *Am J Trop Med Hyg* **2014**, *90* (5), 819-22.
55. Bygott, J. M.; Chiodini, P. L., Praziquantel: neglected drug? Ineffective treatment? Or therapeutic choice in cystic hydatid disease? *Acta Trop* **2009**, *111* (2), 95-101.
56. De, S., et al., In vitro assessment of praziquantel and a novel nanomaterial against protoscoleces of *Echinococcus granulosus*. *J Helminthol* **2012**, *86* (1), 26-9.
57. Moreno, M. J., et al., Praziquantel and albendazole in the combined treatment of experimental hydatid disease. *Parasitol Res* **2001**, *87* (3), 235-8.
58. Horton, R. J., Albendazole in treatment of human cystic echinococcosis: 12 years of experience. *Acta tropica* **1997**, *64* (1-2), 79-93.
59. Rowley, A. H., et al., Albendazole treatment of recurrent echinococcosis. *The Pediatric infectious disease journal* **1988**, *7* (9), 666-7.
60. Beard, T. C., The elimination of echinococcosis from Iceland. *Bulletin of the World Health Organization* **1973**, *48* (6), 653-60.
61. Woollard, D. J., et al., Epitope specificities and antibody responses to the EG95 hydatid vaccine. *Parasite immunology* **1998**, *20* (11), 535-40.

62. Gauci, C. G., et al., Protection against cystic echinococcosis in sheep using an *Escherichia coli*-expressed recombinant antigen (EG95) as a bacterin. *Parasitology* **2022**, *150* (1), 1-3.
63. Petavy, A. F., et al., An oral recombinant vaccine in dogs against *Echinococcus granulosus*, the causative agent of human hydatid disease: a pilot study. *PLoS Negl Trop Dis* **2008**, *2* (1), e125.
64. Zhang, W.; McManus, D. P., Vaccination of dogs against *Echinococcus granulosus*: a means to control hydatid disease? *Trends Parasitol* **2008**, *24* (9), 419-24.
65. Moro, P.; Schantz, P. M., Echinococcosis: a review. *Int J Infect Dis* **2009**, *13* (2), 125-33.
66. Wilson, J. F.; Rausch, R. L., Alveolar hydatid disease. A review of clinical features of 33 indigenous cases of *Echinococcus multilocularis* infection in Alaskan Eskimos. *Am J Trop Med Hyg* **1980**, *29* (6), 1340-55.
67. Schweiger, A., et al., Human alveolar echinococcosis after fox population increase, Switzerland. *Emerg Infect Dis* **2007**, *13* (6), 878-82.
68. Lukaschenko, N. P.; J., Problems of epidemiology and prophylaxis of alveolar (multilocular echinococcosis): a general review-with particular reference to the USSR. *Int* **1971**, *1*, 125-134.
69. Kammerer, W. S.; Schantz, P. M., Echinococcal disease. *Infect Dis Clin North Am* **1993**, *7* (3), 605-18.
70. Eiermann, T. H., et al., HLA and alveolar echinococcosis. *Tissue antigens* **1998**, *52* (2), 124-9.
71. Godot, V., et al., Increased basal production of interleukin-10 by peripheral blood mononuclear cells in human alveolar echinococcosis. *European cytokine network* **1997**, *8* (4), 401-8.
72. Sturm, D., et al., Interleukin-5 is the predominant cytokine produced by peripheral blood mononuclear cells in alveolar echinococcosis. *Infect Immun* **1995**, *63* (5), 1688-97.
73. Didier, D., et al., Hepatic alveolar echinococcosis: correlative US and CT study. *Radiology* **1985**, *154* (1), 179-86.
74. Gottstein, B., et al., Improved primary immunodiagnosis of alveolar echinococcosis in humans by an enzyme-linked immunosorbent assay using the Em2plus antigen. *Journal of clinical microbiology* **1993**, *31* (2), 373-6.
75. Carmena, D., et al., The immunodiagnosis of *Echinococcus multilocularis* infection. *Clin Microbiol Infect* **2007**, *13* (5), 460-75.
76. Schantz, P. M., et al., Effects of albendazole on *Echinococcus multilocularis* infection in the Mongolian jird. *J Infect Dis* **1990**, *162* (6), 1403-7.
77. Eckert, J.; Deplazes, P., Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev* **2004**, *17* (1), 107-35.
78. Reuter, S., et al., *In vitro* activities of itraconazole, methiazole, and nitazoxanide versus *Echinococcus multilocularis* larvae. *Antimicrob Agents Chemother* **2006**, *50* (9), 2966-70.
79. Takahashi, K., et al., Efficacy of anthelmintic baiting of foxes against *Echinococcus multilocularis* in northern Japan. *Vet Parasitol* **2013**, *198* (1-2), 122-6.
80. Schultz, L. V., et al., *Mesocestoides* (Cestoda) infection in a California child. *The Pediatric infectious disease journal* **1992**, *11* (4), 332-4.
81. Fuentes, M. V., et al., Short report: a new case report of human *Mesocestoides* infection in the United States. *Am J Trop Med Hyg* **2003**, *68* (5), 566-7.
82. Walker, M. D.; Zunt, J. R., Neuroparasitic infections: cestodes, trematodes, and protozoans. *Semin Neurol* **2005**, *25* (3), 262-77.
83. Pampiglione, S., et al., Human sparganosis in Italy. Case report and review of the European cases. *APMIS* **2003**, *111* (2), 349-54.
84. Hughes, A. J.; Biggs, B. A., Parasitic worms of the central nervous system: an Australian perspective. *Intern Med J* **2002**, *32* (11), 541-53.
85. Lescano, A. G.; Zunt, J., Other cestodes: sparganosis, coenurosis and *Taenia crassiceps* cysticercosis. *Handb Clin Neurol* **2013**, *114*, 335-45.
86. Kean, B. H., et al., Color Atlas / Text of Ophthalmic Parasitology. 1991; p 195-200.
87. Despommier, D., People, Parasites, and Plowshares. 2014.
88. Oren, Z.; Shai, Y., Mode of action of linear amphipathic alpha-helical antimicrobial peptides. *Biopolymers* **1998**, *47* (6), 451-63.
89. Bechinger, B., Structure and functions of channel-forming peptides: magainins, cecropins, melittin and alamethicin. *The Journal of membrane biology* **1997**, *156* (3), 197-211.

90. Otranto, D.; Eberhard, M. L., Zoonotic helminths affecting the human eye. *Parasit Vectors* **2011**, *4*, 41.
91. Wiwanitkit, V., Ocular sparganosis. *Orbit* **2014**, *33* (6), 474.
92. Phares, K., An unusual host-parasite relationship: the growth hormone-like factor from plerocercoids of *Spirometrid* tapeworms. *International journal for parasitology* **1996**, *26* (6), 575-88.
93. Song, T., et al., CT and MR characteristics of cerebral sparganosis. *AJNR Am J Neuroradiol* **2007**, *28* (9), 1700-5.
94. Cui, J., et al., Serodiagnosis of experimental sparganum infections of mice and human sparganosis by ELISA using ES antigens of *Spirometra mansonii spargana*. *Parasitol Res* **2011**, *108* (6), 1551-6.
95. Liu, L. N., et al., Serodiagnosis of sparganosis by ELISA using recombinant cysteine protease of *Spirometra erinaceieuropaei spargana*. *Parasitol Res* **2015**, *114* (2), 753-7.
96. Tan, T. Y., et al., Cerebral sparganosis: case report. *Changgeng yi xue za zhi / Changgeng ji nian yi yuan = Chang Gung medical journal / Chang Gung Memorial Hospital* **1999**, *22* (2), 287-92.
97. Chang, J. H., et al., Subcutaneous sparganosis--a case report and a review of human sparganosis in Taiwan. *The Kaohsiung journal of medical sciences* **1999**, *15* (9), 567-71.
98. El-On, J., et al., *Taenia multiceps*: a rare human cestode infection in Israel. *Vet Ital* **2008**, *44* (4), 621-31.

VII. The Trematodes

The class Trematoda, in the phylum Platyhelminthes, consists of two orders, Monogenea and Digenea. All are parasitic, but only the Digenea, which include the blood, intestinal, and tissue flukes, contain those parasites that infect the human host. Snails are the obligate intermediate hosts for all Digenid trematode species; most are aquatic organisms. These infections are mainly found throughout the tropics and subtropics, while a few are also encountered in temperate zones.¹

Trematodes undergo multi-step developmental cycles in their intermediate (snail) and definitive hosts. As complex as trematode biology is (e.g., many of them have reservoir hosts), potentially offering numerous opportunities for interrupting their life cycles, eliminating medically important species has occurred only to a limited extent and in specific geographic areas: *Schistosoma mansoni* in multiple Caribbean islands of St. Maarten and St. Kitts, and *S. japonicum* in Japan.² However, progress is being made toward eliminating *S. japonicum* in China. Counterbalancing this good news is the reality that due to climate change and other 21st-century forces, schistosomiasis may be re-emerging in different parts of the African continent, and it has been noted that schistosomiasis has emerged in Corsica, France.³

Trematodes maintain their site location within the host using two suckers, one anterior and one ventral. The anterior sucker also serves as the opening to the oral cavity, into which host tissues are ingested. The outer surface of the adult is covered with a tegument similar in design and function to the tegument of cestodes. It serves as an absorbing surface for both large and small molecular-weight compounds. Membrane-bound microvilli cover the tegument and contain the mitochondria,

pinocytotic vesicles, and other structures facilitating nutrient acquisition.

In addition, trematodes have a functional, bifurcated, blind (i.e., dead-end) gut into which they ingest host tissues. Ingested material is pumped into the intestinal tract, where parasite enzymes (e.g., proteases, lipases, aminopeptidases, esterases) are used for digestion. Since the gut has no exit, wastes are regurgitated into the host. Since trematodes obtain various nutrients in several ways, developing drugs or immune-based therapies explicitly designed to interrupt metabolic processes has proven challenging.

Several layers of muscle lie just below the tegument, allowing them to move about freely within their host. This activity can result in severe pathological consequences, particularly in the case of *Fasciola hepatica*, *Paragonimus westermani*, and the schistosomes.

A pair of dorsal ganglia (i.e., a primitive brain) gives rise to bilateral peripheral nerves running the length of the body, innervating the muscle layers. Commissures from the lateral nerves also innervate various organs, including the gut and reproductive organs. The flukes have no body cavity; organs are embedded within the parenchyma.

In addition to solid wastes, trematodes excrete numerous small molecular-weight compounds facilitated by tubules connecting to collecting organs. These, in turn, connect to the excretory pore at the tegumental surface of the parasite.

Genetic analysis of whole genome sequences of many species of human-infecting trematodes showed that they employ aerobic metabolic pathways to process glucose.⁴

Trematodes in the order Digenea make use of one of three reproductive strategies: 1. self-fertilization, in which the same worm possesses both sets of reproductive organs (e.g., *Fasciola hepatica*), 2. cross-fertilization between two worms possessing both sets of reproductive organs (e.g., *Paragonimus westermani*), or 3. fertilization between worms of the opposite sex, as is the case among the schistosomes.

Egg production is a process that employs a series of specialized organs. The ovum, supplied with yolk from the vitelline glands, is fertilized within the oviduct and becomes surrounded by a shell from the Mehlis' gland secretions. The unembryonated, fully formed, fertilized eggs exit from the parasite through the genital pore, usually situated in between the anterior and ventral suckers.

Once the eggs reach freshwater, or, for some species, a suitable terrestrial niche, they embryonate and are either stimulated to hatch in the external environment or hatch after being ingested by the appropriate snail intermediate host. Each species of trematode has evolved to infect specific species of snail hosts. This offers hope of controlling a few trematode infections simply by eliminating their snail hosts. Once inside the snail, the trematode embryo undergoes asexual replication, eventually increasing in numbers and transforming into cercariae. Infection in the snail rarely results in the death of the mollusk. The cercaria then penetrates out of the snail and continues their life cycle. Other invertebrates (e.g., insects, crabs) and various cold-blooded vertebrates (e.g., fish) function as secondary hosts for some medically important species of trematodes. For some, freshwater plants (e.g., watercress, water chestnuts) are sites onto which the cercaria attaches, transforming into the environmentally resistant metacercaria, the infectious stage of the parasite. Reservoir hosts exist for many trematode species,

making their control much more complex than other infectious agents that only infect the human host.

The infectious stage is the cercaria for a few trematode species, most notably the schistosomes. A cercaria can actively swim in fresh water for 12-14 hours in search of a human host before using its energy supply.⁵ It stalks its host by following a trail of lipids, peptides, and arginine that elutes from our skin.⁶ When a swimmer, fisherman, or rice farmer comes in contact with water containing cercaria, the cercaria can enter the host by penetrating a hair follicle.

The physiological and biochemical aspects of site selection by trematodes within their human hosts still need to be better understood. It is known that it results from a complex interplay between chemical and physical niches, which comprise the spectrum of environmental cues from the host and the receiving and translation of those cues by the parasite's nervous system. Some drugs interfere with trematode nervous system functions (e.g., praziquantel), resulting in profound, often lethal, changes in worm behavior. Emerging data finds that trematodes can also induce neoplastic changes leading to cancer, including cholangiocarcinoma resulting from either clonorchiasis or opisthorchiasis and bladder cancer from the urogenital form of schistosomiasis.⁷

Regrettably, mass drug treatment with praziquantel for schistosomiasis once touted as a panacea for the global control of this insidious disease, has not proven as effective as initially hoped, despite decades of use and currently hundreds of millions of people treated yearly. An integrated approach of sanitation, snail control, and mass praziquantel administration to advance disease elimination efforts is part of the recommendations of the WHO 2020 NTD Road map.⁸ The plan addresses all

aspects of integrated control for 2021–2030, including finances, implementation, monitoring, and evaluation of the recommended interventions, starting at the community level and reaching global efforts.⁸ This comprehensive set of guidance, with programmatic recommendations, is designed to catalyze new efforts towards the goal of a world free of NTDs by 2030.⁸ Some countries have made progress in addressing the vicious cycle of tropical diseases in low-income countries; for others, the 2020 WHO NTD road map

provides guidance to enable coordination of local and global efforts to attain specific disease elimination targets and progress towards achieving sustainable development goals. Due to climate change and other modern forces, it may be necessary to implement added schistosomiasis control tools, including combination treatment with praziquantel and synergistic antimalarial drugs or new schistosomiasis vaccines now undergoing phase 1–2 clinical trials.^{9,10}

References

1. Chai, J. Y.; Jung, B. K., General overview of the current status of human foodborne trematodiasis. *Parasitology* **2022**, *149* (10), 1262–1285.
2. Hewitt, R.; Willingham, A. L., Status of Schistosomiasis Elimination in the Caribbean Region. *Trop Med Infect Dis* **2019**, *4* (1).
3. Blum, A. J.; Hotez, P. J., Global “worming”: Climate change and its projected general impact on human helminth infections. *PLoS Negl Trop Dis* **2018**, *12* (7), e0006370.
4. Biswal, D. K., et al., De novo genome and transcriptome analyses provide insights into the biology of the trematode human parasite *Fasciolopsis buski*. *PloS one* **2018**, *13* (10), e0205570.
5. Whitfield, P. J., et al., Age-dependent survival and infectivity of *Schistosoma mansoni* cercariae. *Parasitology* **2003**, *127* (Pt 1), 29–35.
6. Haeberlein, S.; Haas, W., Chemical attractants of human skin for swimming *Schistosoma mansoni* cercariae. *Parasitol Res* **2008**, *102* (4), 657–62.
7. Prasopdee, S., et al., Update on the risk factors for opisthorchiasis and cholangiocarcinoma in Thailand. *Parasites Hosts Dis* **2023**, *61* (4), 463–470.
8. Organization, W. H., Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva. *Licence: CC BY-NC-SA 3.0 IGO* **2020**.
9. Mnkugwe, R. H., et al., Efficacy and safety of praziquantel and dihydroartemisinin piperaquine combination for treatment and control of intestinal schistosomiasis: A randomized, non-inferiority clinical trial. *PLoS Negl Trop Dis* **2020**, *14* (9), e0008619.
10. Hotez, P. J., et al., Advancing the Development of a Human Schistosomiasis Vaccine. *Trends Parasitol* **2019**, *35* (2), 104–108.

33. The Schistosomes

Pronunciation: \shis-tə-'sō-mə\

Schistosoma mansoni

(Sambon 1907)

Pronunciation: \man-sə-,nī\

Schistosoma japonicum

(Katsurada 1904)

Pronunciation: \jə-pān-c-əm\

Schistosoma haematobium

(Bilharz 1852)

Pronunciation: \hē-mə-'tō-bē-əm\

Schistosoma mekongi

(Bilharz 1852)

Pronunciation: \mə-kāŋ-ē\

Schistosoma intercalatum

(Fischer 1934)

Pronunciation: \in-tər-kə-la-təm\

Introduction

Five digenean trematode species in the genus *Schistosoma* (SHIS-tih-sow-mah\), *S. mansoni* (MAN-sow-nigh\), *S. haematobium* (HEE-mah-tow-BEE-um\), *S. japonicum* (JIH-pon-ik-um\), *S. mekongi* (MUH-kon-ik-um\), and *S. intercalatum* (IN-ter-cal-AH-

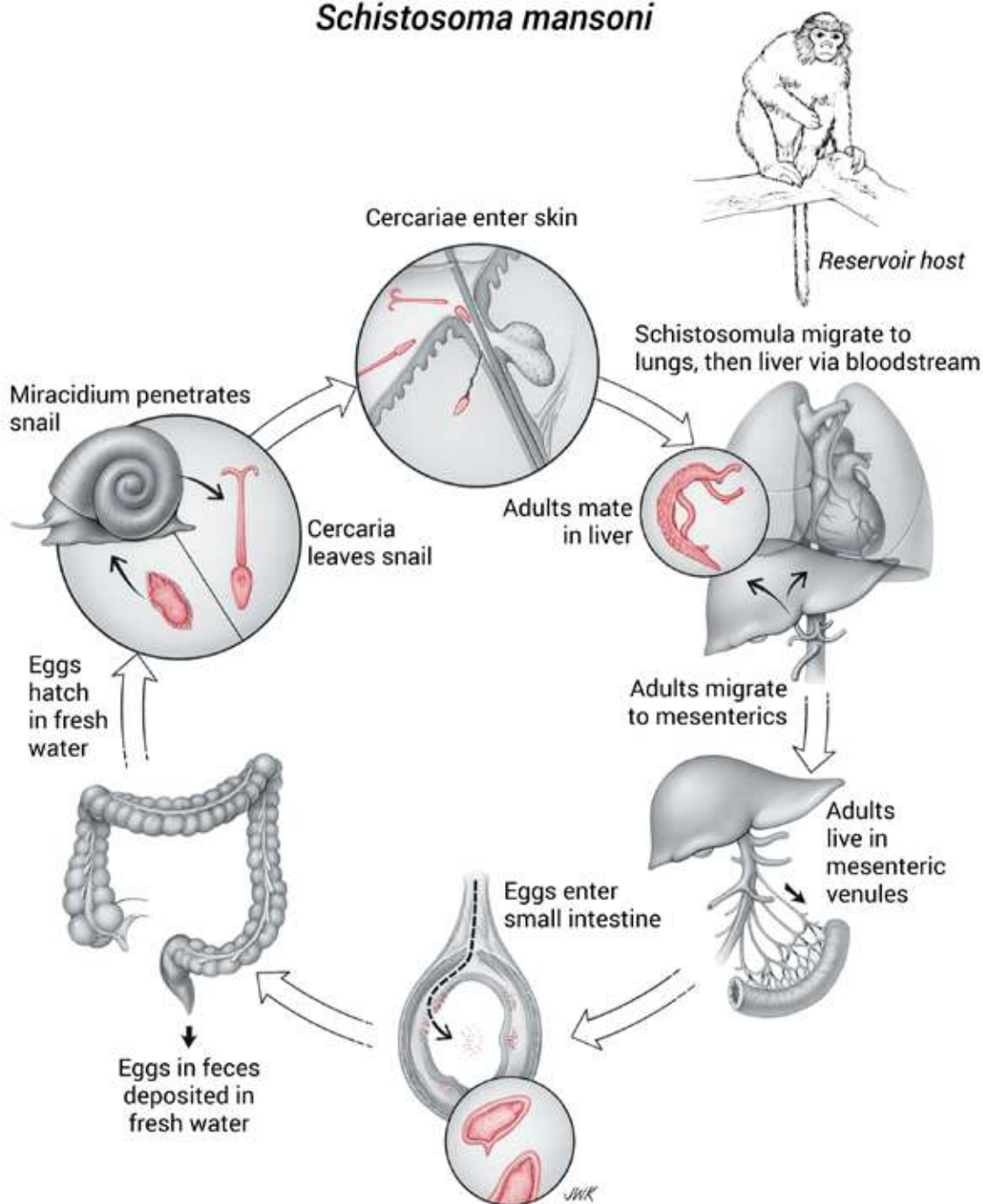


Figure 33.1. Scanning electron micrograph of *Schistosoma mansoni* adults. (From Kessel and Shih: Scanning Electron Microscopy in Biology. Springer-Verlag, 1976. Reproduced with permission).

tum\), are responsible for a series of related water-borne infections in humans. The clinical diseases they cause are collectively referred to as schistosomiasis. *S. intercalatum*, a parasite primarily of cattle in West Africa, is also a rare human infection. All the above species use a variety of freshwater snails as their intermediate hosts. Infection is spread from human to human by exposure to tropical and subtropical freshwater ecosystems contaminated with untreated urine and feces containing Schistosome eggs.

S. haematobium infects the venules surrounding the bladder and is the causative agent of urinary tract disease and female genital schistosomiasis (FGS). The remaining four species infect the venules of the small and large intestine. Regardless of the species, the bulk of pathological consequences are caused by their eggs, many of which locate to the capillary beds of all organs of the infected host and block circulation. Chronic schistosomiasis is responsible for physical growth and cognitive delays in children.¹ The Global Burden of Disease Study in 2013 estimated that almost 300 million people suffer from some form of schistosomiasis, with approximately 90% of the cases found in Africa.² The 2020 WHO NTD roadmap estimates of disease burden are 24,000 deaths in 2016 with 2.5 million DALY's and approximately 236 million people requiring mass drug treatment.² The Global Burden of Disease Study 2019 estimates that 140 million people live with schistosomiasis, resulting in 1.64 million DALYs and 11,500 deaths.³ Others find the numbers of cases, DALYs, and fatalities are undercounted and, in fact, several-fold higher.⁴ Poor sanitation and poverty are critical factors, as they are for many human parasitic diseases.⁴ Forced migration of people due to armed conflict throughout many parts of the world, particularly Africa, and encroachment into natural systems (e.g., constructing irrigation canals and dams) have resulted in

Schistosoma mansoni



regional increases in schistosomiasis.

S. mansoni is found throughout most of tropical Sub-Saharan Africa, Egypt, and Sudan, parts of the Middle East, some parts of South America (including Brazil, Venezuela, and the Guyanas), and a few islands or countries in the Caribbean, including Suriname and St. Lucia. It is the only Schistosome species found in the New World and was most likely first introduced there several hundred years ago through the middle passage of the Atlantic slave trade.⁵ At one time, schistosomiasis was endemic in Puerto Rico and other Caribbean islands, but these foci are being eliminated.⁶ Its intermediate hosts are aquatic snails in the genus *Biomphalaria*. Other hosts for *S. mansoni* include baboons and monkeys in Africa. They play no significant role in the epidemiology of human disease and are not considered reservoirs for the infection.

S. haematobium is prevalent in most parts of Africa and some parts of the Middle East. Its aquatic intermediate host snails are in the genus *Bulinus*. There are no medically important reservoir hosts for this trematode species either. During an isolated outbreak among white-water rafters in the Omo River Valley of Ethiopia, the outbreak's origin was traced back to monkeys.⁷ An outbreak of *S. haematobium* was also reported in Corsica, France, most likely initiated by an infected individual from Africa who had migrated there.^{8,9} However, subsequent studies found that transmission is ongoing, and schistosomiasis has emerged.¹⁰ The schistosome species that caused the outbreak may represent a hybrid of *S. haematobium* and *S. bovis* adapted to the local snail population to survive winter without an animal reservoir. *S. bovis* is capable of human infection, updating the previous belief that it only infected non-humans.¹¹ A 2023 review covers the molecular origins and extent of hybridization across *S. haematobium* and putative non-human

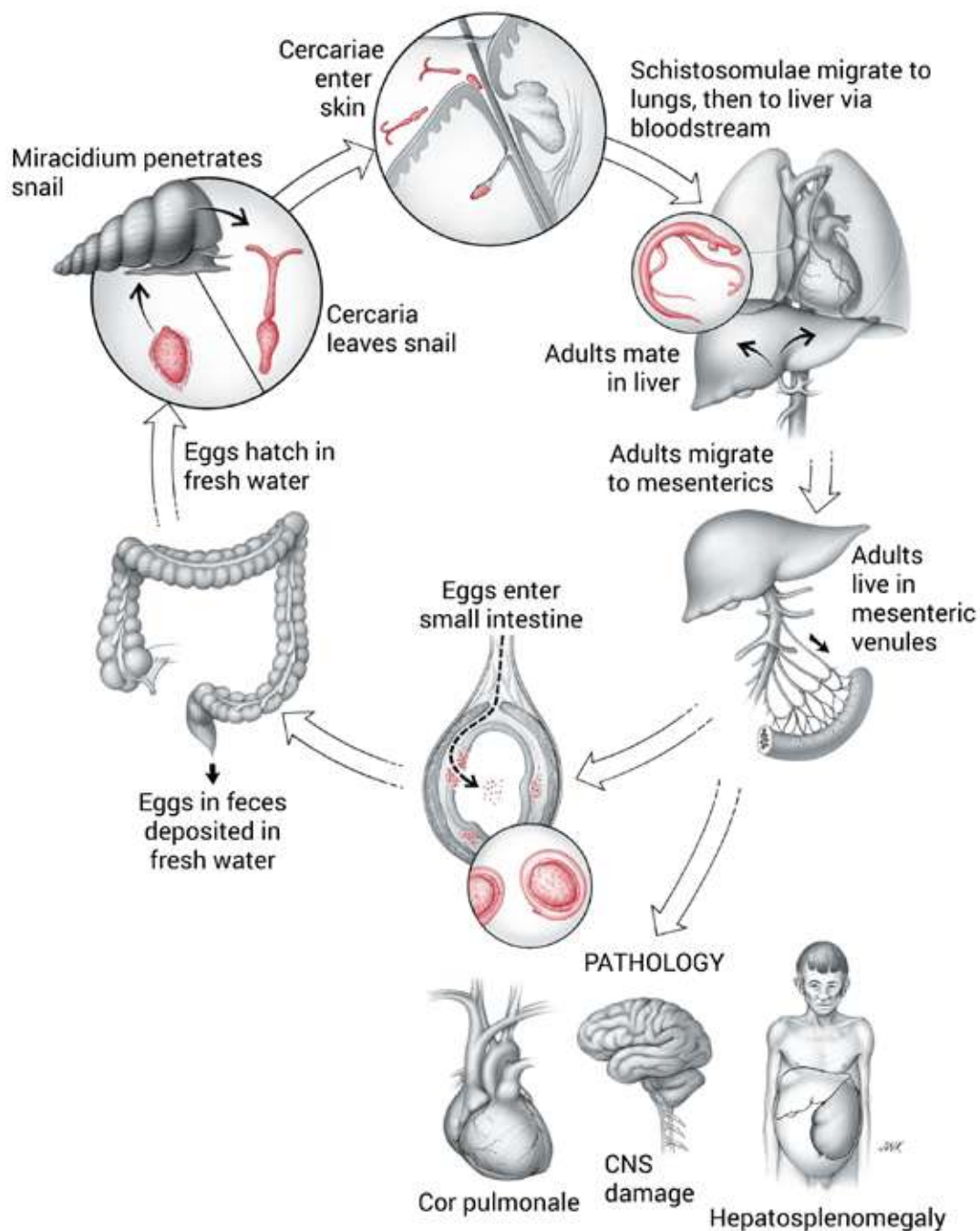
species in greater detail.¹² Previously known as urinary tract schistosomiasis, increasing evidence finds that *S. haematobium* is also a significant cause of female genital schistosomiasis, sometimes referred to as urogenital schistosomiasis. Urogenital schistosomiasis has emerged as a significant gynecologic condition on the African continent.¹³ In East and South African countries such as Tanzania, Zimbabwe, Malawi, Mozambique, and KwaZulu-Natal South Africa, urogenital schistosomiasis is a significant co-factor in the HIV/AIDS epidemic.¹⁴ *S. intercalatum* occasionally infects people in Cameroon, Gabon, and the Democratic Republic of Congo.¹⁵⁻¹⁷

S. japonicum occurs in China, the Philippines, and Indonesia. It was eliminated from Japan in 1977 and is declining significantly in China, leaving the Philippines as a significant source of *S. japonicum* infection. Its



Figure 33.2. Scanning electron micrograph of adult schistosomes. Notice gynecophoral canal with female inside. Photo D. Scharf.

Schistosoma japonicum



amphibious intermediate host snails are in the genus *Oncomelania*. In contrast to the other schistosomes, zoonotic transmission of *S. japonicum* occurs frequently. There are important reservoir hosts for *S. japonicum*, including water buffalo, cattle, non-human primates, and pigs.²⁰ *S. mekongi*, a closely related species, is found throughout the Mekong River and its delta in South-east Asia. Although similar in morphology and life cycle to *S. japonicum*, *S. mekongi* is genetically distinct.²¹

There are no autochthonous infections in the United States with any of the above species of schistosomes because there are no appropriate species of intermediate host snails, and, most importantly, sanitary disposal of feces and urine is the general rule. Thousands of Caribbean, African, and Southeast Asian immigrants and refugees may be infected, so clinicians who practice only in nonendemic areas must still be knowledgeable regarding this parasitic infection that causes a high burden of morbidity and mortality.

Historical Information

Molecular genomic studies indicate that the schistosomes originated in Asia, with the first clade of *S. japonicum* likely derived from avian schistosomes that first infected rodents before humans.²² *S. mansoni* and *S. haematobium* represent two distinct clades that dominate the African continent. Paleo-parasitologists have found *S. haematobium* eggs in mummies dating as far back as 1250 BCE.²³ It is speculated that ancient Egyptians believed the advent of manhood was heralded by the appearance of blood in the urine (hematuria), analogous to the onset of menstruation in women. Still, there remains controversy about this despite possible references to *Schistosoma*-induced hematuria in early Egyptian papyri.²⁴ Hematuria in males represents a late manifestation of *S. haema-*

tobium infection.

In 1798, A. J. Renoult, a French army surgeon, recorded the first modern description of hematuria due to *S. haematobium*, chronicling the epidemic of hematuria experienced by Napoleon's soldiers who invaded Egypt.²⁵ Cases were subsequently described among troops involved in the Second Anglo-Boer War (1899–1902).²⁶ In 1851 and 1852, Theodore Bilharz and Ernst von Siebold reported on the first human cases of *S. haematobium*, described the adult worm, and made the connection with the appearance of hematuria.^{27–29} They identified this as a parasite that occupied the venous plexus of the bladder and whose eggs possessed a terminal spine. Two years later, Wilhelm Griesinger described the clinical disease and its pathology in detail.³⁰ He noted the relation of the infection to the involvement of the bladder and ureters. In 1918, Robert Leiper described the life cycle of *S. haematobium*, its intermediate host, and its morphology.³¹ He also carried out experimental infections with *S. haematobium* in various indigenous animals of northern Egypt, proving that rats and mice were susceptible.

Although the symptoms associated with *S. haematobium* are unambiguous in early writings, other forms of schistosomiasis came to be recognized and understood. In 1902, Patrick Manson described a case of schistosomiasis in an Englishman who had traveled extensively throughout the Caribbean. He found many eggs with lateral spines in the patient's stool but not in his urine.³² Five years after Manson's case description, Louis Sambon recognized two blood flukes based on morphology and origin of the eggs in stool and urine.³³ In tribute to Manson, Sambon named this new organism after him, *Schistosoma mansoni*.²⁹ In 1908, Piraja de Silva described cases of *S. mansoni* in South America.³⁴ By 1918, Leiper had conducted

Schistosoma haematobium

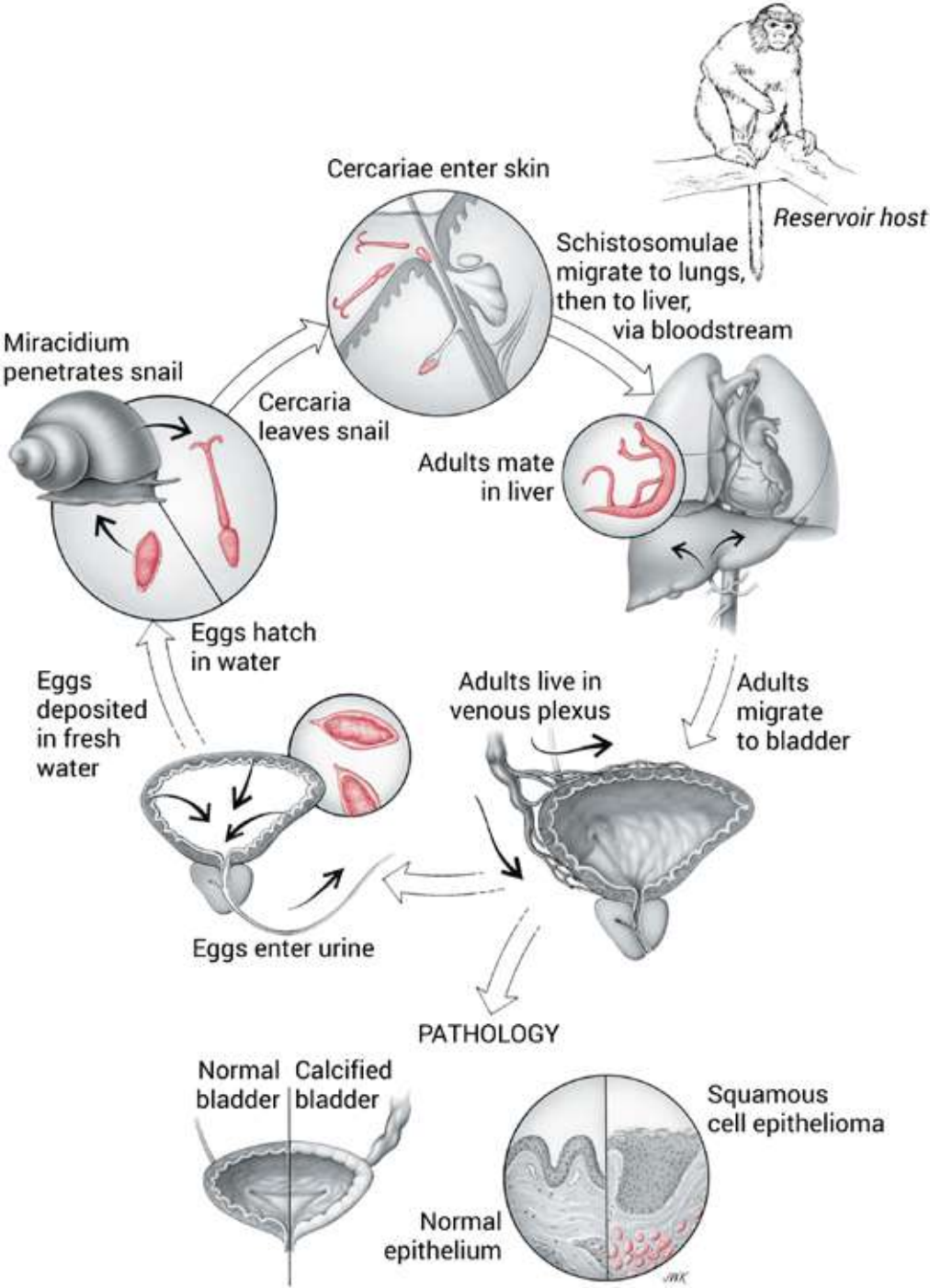




Figure 33.3a. Adult schistosomes *in situ*. The elongated worms appear dark due to the ingestion of hemoglobin.

extensive investigations on schistosomiasis and reported the life cycle of *S. mansoni*, in which he described its snail intermediate host and the morphology of the adult worms.³¹

Giving credit for the discovery of *S. japonicum* is more involved. In 1888, Tokuho Majima described a case of cirrhosis and linked the presence of *S. japonicum* with this disease.³⁵ In 1904, Fujiro Katsurada described *S. japonicum* adult worms from infected cats.³⁶ At the same time, John Catto, working in Singapore, described an identical adult worm in a cholera patient at autopsy.³⁷ Catto named it *S. cattoi*, but his publication was delayed, and the name *S. japonicum* was accepted instead. In 1904, Kenji Kawanishi made the correlation between the clinical

condition, Katayama fever (acute schistosomiasis), and the presence of *S. japonicum* adults after finding eggs of this parasite in the stools of patients suffering from the acute phase of the infection.³⁸ Kan Fujinami and Hatchitaro Nakamura in 1909, and Yoenji Miyagawa three years later independently reported similarly detailed life cycle descriptions.^{39, 40} In 1914, Keinosuke Miyairi and Masatsugu Suzuki identified various snails in the genus *Oncomelania* as the intermediate hosts.⁴¹

S. japonicum infection has significantly impacted the history of modern China. Mao's troops could not launch an amphibious assault on Taiwan in the late 1940s because they developed Katayama fever while encamped along the Yangtze River.⁴² Later on, during the Great Leap Forward, Mao mobilized tens of thousands of workers to either bury *Oncomelania* snails along the clay banks of rice paddy irrigation canals or remove them by hand, one at a time.⁴³ This proved to be an effective control measure whose positive effects of reducing the infection rate in rice

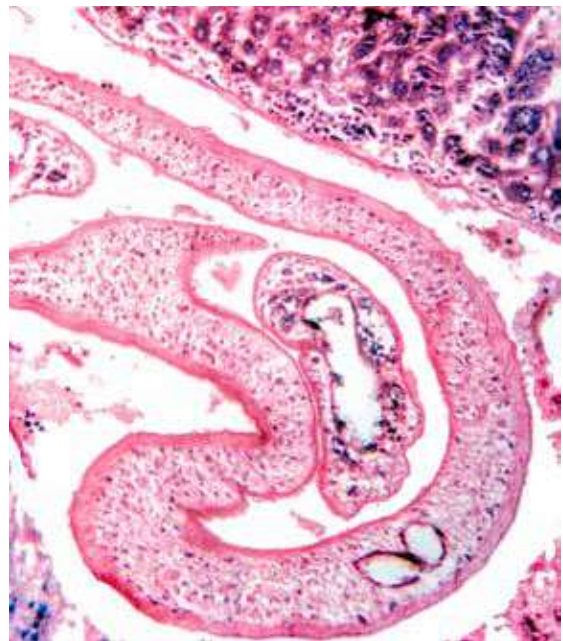


Figure 33.3b. Cross section of a pair of adult schistosomes *in situ* in a mesenteric venule.

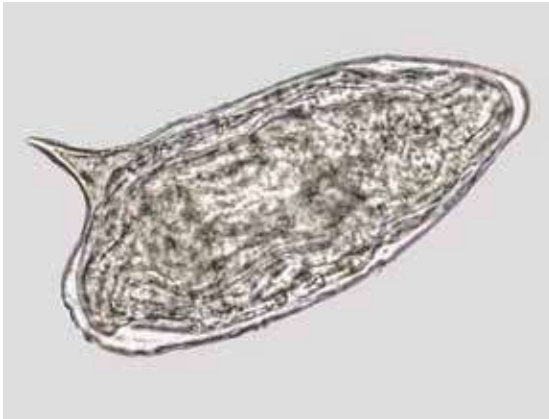


Figure 33.4. Egg of *Schistosoma mansoni*. Note lateral spine. 150 μm x 60 μm .

farmers can still be appreciated today.

In the 1900s, some additional species of schistosomes, including *S. mekongi* and *S. intercalatum*, were discovered, and their life cycles, specific snail intermediates, and disease manifestations were described.⁴⁴

Life Cycle

Unlike most digenean trematodes, Schistosomes have separate sexes (Fig. 33.1); the female measures 15 mm long, and the male measures 10 mm. Schistosome adults remain *in copula* (Fig. 33.2) for most of their life span, living attached by their oral and ventral sucker disks to the endothelium of venules (Figs. 33.3a, 33.3b). *S. mansoni* lives in the inferior mesenteric veins that drain the intes-

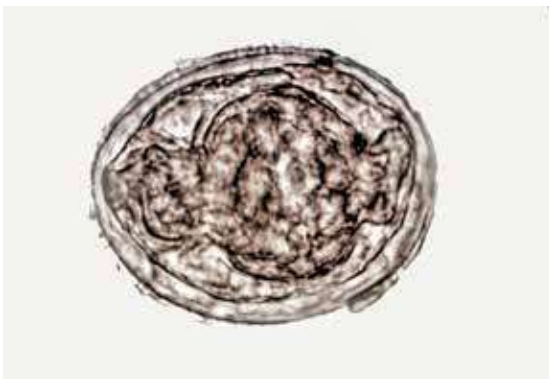


Figure 33.5a. Egg of *Schistosoma japonicum*. No spine can be seen. 85 μm x 60 μm .

tine, while *S. japonicum* and *S. mekongi* live in the superior mesenteric veins. *S. japonicum* adult worms can also find their way to the choroid plexus, the venules around the spinal column, and other ectopic locations. *S. haematobium* is found almost exclusively in the venous plexus, which drains the urinary bladder. The routes by which adult schistosomes arrive at these sites have been studied for various species using several animal models and are known to involve migration through various capillary beds.⁴⁵

Adult worms live 5–8 years on average, although some have lived as long as 37 years.⁴⁶ Schistosomes are facultative anaerobes that derive energy primarily through the degradation of glucose and glycogen. They utilize tegument-associated transport mechanisms for glucose uptake and utilization.⁴⁷

Adult schistosomes metabolize hemoglobin ingested into their blind, bifurcated gut as a primary source of amino acids.³⁰ They



Figure 33.5b. Egg of *Schistosoma mekongi*. No spine can be seen. 65 μm x 50 μm .

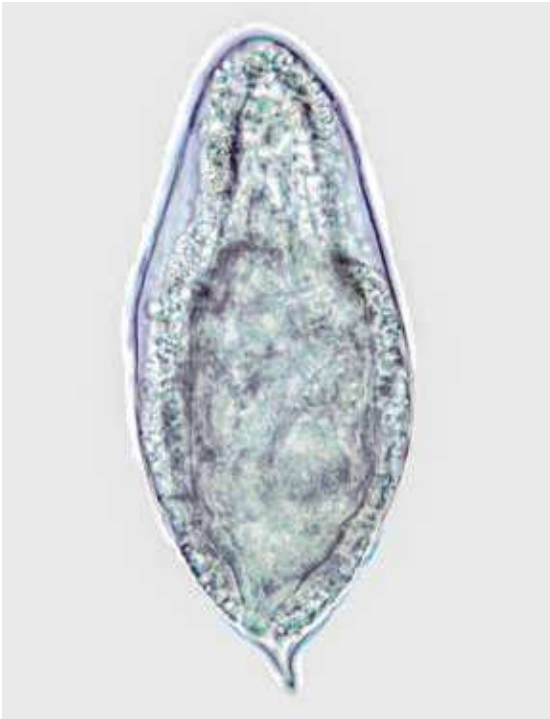


Figure 33.6. Egg of *Schistosoma haematobium*. Note terminal spine. 155 μm x 55 μm .

employ a hemoglobinase, digesting the globin portion of the molecule and detoxifying the heme moiety into a pigment before it is regurgitated back into the bloodstream.⁴⁸ The female lies within the gynecophoral canal of the male (Fig. 33.2). This muscular, tegumental fold extends down both sides of

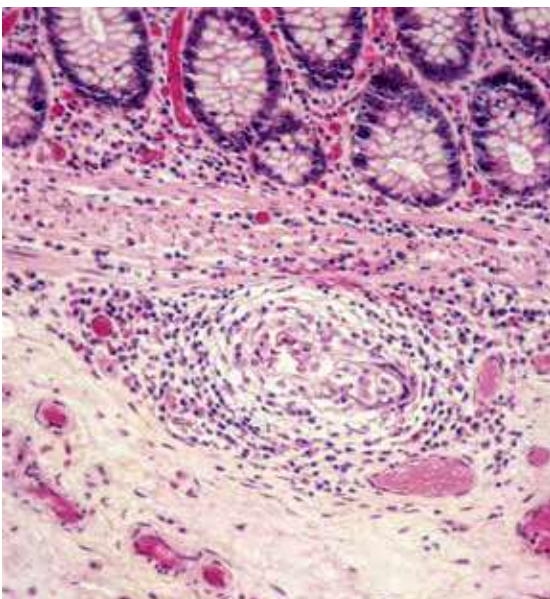


Figure 33.7. *Schistosoma* egg in tissue of the small intestine. Note intense granuloma.

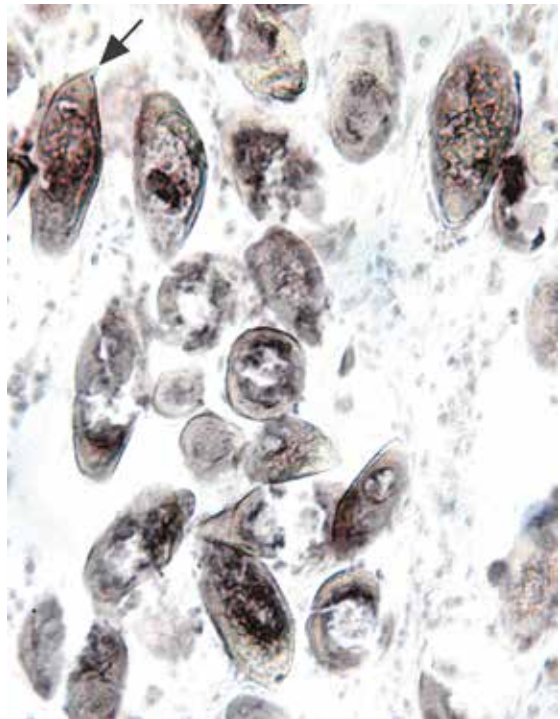


Figure 33.8. *S. haematobium* eggs in bladder wall. Note terminal spine (arrow).

the male and may enable the female to feed on blood by assisting in pumping blood into their esophagus.

Active transport mechanisms transport free amino acids and glucose across the tegument. They store excess glucose as glycogen. The tegumental surface of male *S. mansoni* is covered with finger-like projections, termed “papillae,” while male *S. haematobium* has more widely spaced, shorter, finger-like projections, termed “tubercles.” Evidence suggests that these projections have a sensory function.⁴⁹

Once the worms pair off and migrate from the liver to the venules, the females receive a dipeptide pheromone from the males (β -alanyl-tryptamine) that is required for egg production.⁵⁰ Experimental infections with females, or females separated from males, then re-introduced into the same host, cannot produce eggs. Females remain *in copula* with a male partner and lay eggs throughout their lifespan. The eggs of *S. mansoni* are oval and



Figure 33.9. Miracidium of *S. mansoni* caught in the act of hatching.

possess a lateral spine (Fig. 33.4); those of *S. japonicum* and *S. mekongi* are globular and have a small inconspicuous spine (Fig. 33.5a, 33.5b); eggs of *S. haematobium* are oval,



Figure 33.10. Miracidium of *S. mansoni*. Phase contrast.



Figure 33.11. *Biomphalaria glabrata*, the most common intermediate snail host for *S. mansoni*.

with a terminal spine (Fig. 33.6). *S. mansoni* females produce, on average, 300 eggs per day, while *S. japonicum* and *S. mekongi* shed 1500–3000 eggs daily. *S. haematobium* female worms produce hundreds of eggs per day.⁵¹

When the female worm applies her ventral sucker to the endothelial surface, eggs pass through the birth pore located above the ventral sucker, encounter endothelial cells, and



Figure 33.12. *Oncomelania nosophora*, a snail intermediate host for *S. japonicum*.

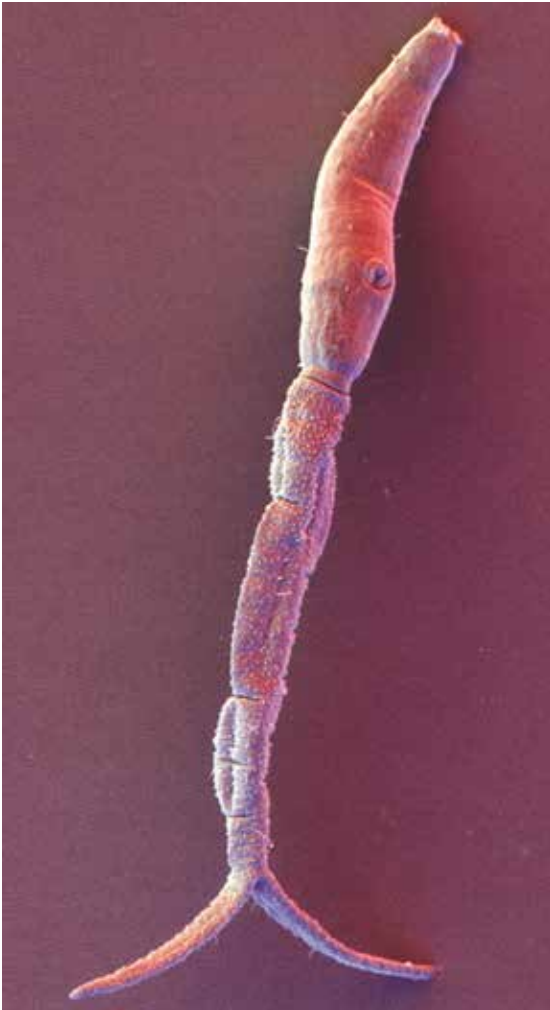


Figure 33.13. Scanning electron micrograph of a cercaria of *S. mansoni*. Photo D. Scharf.

penetrate the surrounding connective tissue. The larva (miracidium) in the egg secretes proteolytic enzymes facilitating this process. Eggs collect in the sub-mucosa (Fig. 33.7) before lysing their way into the lumen of the small intestine or, in the case of *S. haematobium*, the lumen of the bladder.

When adult females raise their ventral suckers from the surface of the endothelium, eggs inadvertently escape into the circulation, carrying them to the liver via the portal circulation. Nearly 50% of all *S. mansoni* eggs produced end up in the liver, a dead end for the life cycle. Eggs that reach the lumen of the small intestine are included in the fecal mass.

Eggs of *S. haematobium* must traverse the wall of the bladder (Fig. 33.8) before exiting the host in the urine. In heavy infections, the damage caused by the eggs to the intestinal or bladder wall can lead to clinical disease.

For the life cycle to continue, eggs in feces or urine must be deposited in freshwater. There, environmental cues trigger the miracidium to hatch (Fig. 33.9). This ciliated, free-swimming stage (Fig. 33.10) seeks out its appropriate snail intermediate host, relying on a gradient of appropriate low molecular weight signals emanating from the snail host to do so. In essence, the snail is a chemical homing device for the parasite. The process is called chemotaxis.

Upon finding its host snail (Figs. 33.11, 33.12), the miracidium penetrates the soft, fleshy foot, facilitated by a different set of proteolytic enzymes than it used to exit from the mammalian host. The details of parasite replication leading to the production of the cercaria, the infectious stage for all Schistosome species, is poorly understood due to its rapid progression from one stage to the next inside the snail. The miracidium invades the snail and transforms into a primary (mother) sporocyst near the penetration site. The primary sporocyst produces secondary (daughter) sporocysts by asexual reproduction, and this stage migrates from the foot to the hepatopancreas. Daughter sporocysts produce the cercariae, most of which (over 94 percent) are shed during the daytime. Each miracidium is either male or female, as are the resulting cercariae. For each stage of development, there is a dramatic increase in the number of organisms. A single miracidium of *S. mansoni* produces some 4,000 genetically identical cercariae (Fig. 33.13). The snail remains alive, even if infected with many miracidia.

Cercariae exit from the snail aided by yet another set of proteolytic enzymes. Cercar-

iae are positively phototropic and negatively geotropic. The forked-tailed cercariae accumulate at the surface of freshwater ecosystems and stalk their definitive host by swimming towards increasing concentrations of chemical cues, including linoleic acid, a fatty acid found on the entire surface of human skin. Cercariae must infect its definitive host within 8 hours after emerging from its snail host; otherwise, they exhaust their glycogen reserves and die.

Infection in the human host begins when the cercariae penetrate unbroken skin. With *S. mansoni*, this step requires about half an hour and occurs more rapidly with *S. japonicum*.⁵² Skin penetration is usually through a hair follicle and is facilitated by releasing a new set of proteases and eicosanoids.⁵³ Cercariae shed their forked tails during penetration and rapidly transform within the dermal layer of skin into the schistosomula stage. After approximately two days, the schistosomulae leave the dermis via the circulation and end up in the lungs' capillaries, where they remain for several more days. Here, the immature worms acquire the ability to incorporate host serum proteins onto their tegumental surface. This "camouflage" (a cloaking device, if you will) allows the schistosomula to evade the immune system, enabling the parasite to live "long and prosper." In addition, the worm possesses a β -2-microglobulin-like molecule that aids in confusing immune defense cells, particularly macrophages, in their attempt to recognize parasite antigens. Schistosomulae migrate from the lungs via the circulation to the liver, where they mature into adult worms. Both sexes produce mutually attractive pheromones, and eventually, worms of the opposite sex find each other in the vastness of the parenchymal tissue. After mating, the newlyweds migrate out into the mesenteric circulation *in copula*. *S. mansoni* and *S. japonicum* worm pairs live in the mesenteric venules that drain the intestines. In contrast, *S. haemato-*

bium worm pairs live in the venous plexus of the bladder and possibly small venules that drain the female genital tract. Egg production begins shortly after that. Other mammalian species, including baboons, rhesus monkeys, chimpanzees, mice, and rats, can be experimentally infected with the cercariae of *S. mansoni*. Few viable eggs are produced in the rat, however.

Cellular and Molecular Pathogenesis

The larval forms and adult worms do not cause disease or significant pathological damage, living in harmony with the host by simply avoiding its immune system.^{54, 55} In addition to their unique cloaking device⁵⁰, they routinely shed tegumental components, avoiding long-term exposure to host immune surveillance systems.⁵⁶ Adult schistosomes in the venous circulation can harbor enteric bacteria affixed to their surface. This relationship can result in enteric bacteria, such

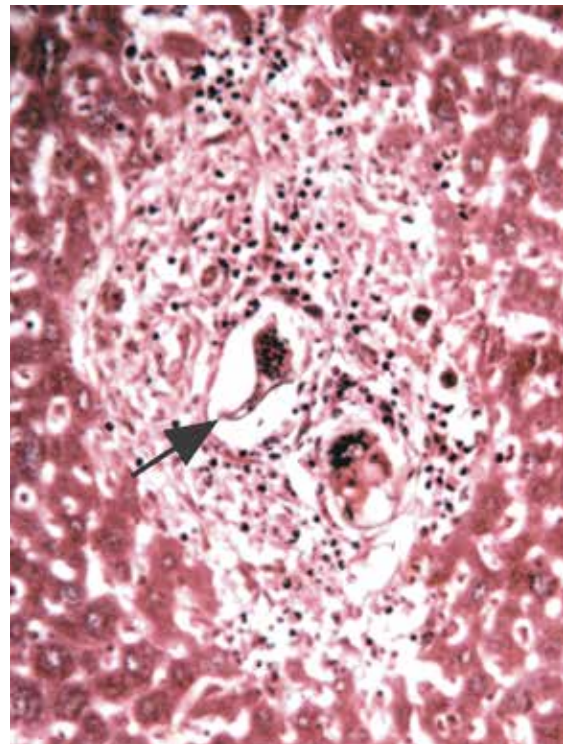


Figure 33.14. Granuloma in liver surrounding eggs of *S. mansoni*. Note the lateral spine (arrow).

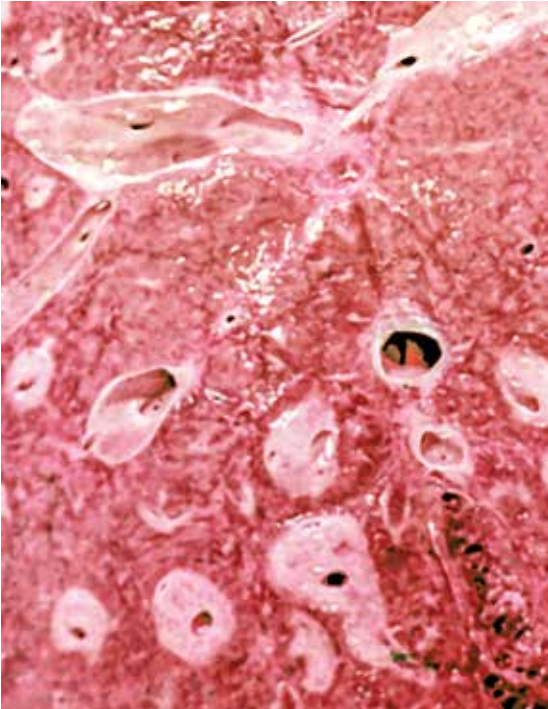


Figure 33.15. Pipe stem fibrosis in liver due to heavy infection with *S. mansoni*. Note normal liver tissue next to fibrotic vessels.

as *Salmonella*, being introduced directly into the bloodstream. As a result, there is a well-described association between chronic schistosomiasis and so-called enteric fevers from non-typhoidal salmonellosis.⁵⁷

In contrast to adults, the eggs produced by the female worm result in profound immunopathologic responses. This phenomenon accounts for almost all of the worm-induced pathology and clinical manifestations. For *S. japonicum* and *S. mansoni*, egg deposition occurs in the circulation of the small intestine and liver (Fig. 33.14) to produce intestinal and hepatic fibrosis, whereas *S. haematobium* egg deposition occurs in the circulation of the bladder to produce fibrosis, leading in many cases to an obstructive uropathy. Heavy egg deposition occurs predominantly in individuals with large numbers of adult worms.

Clinical illness caused by schistosomes generally occurs only in people who suffer from recurrent heavy worm burdens. Increasing



Figure 33.16. X-ray showing calcified dome of the bladder due to chronic infection with *S. haematobium*.

evidence suggests that a component of this phenomenon depends on host genetic factors.⁵⁸ In this regard, the same genes specific for susceptibility to *S. mansoni* have been identified in people living in Africa and South America.⁵⁹ In a study in Sudan, a specific gene locus correlated with advanced liver disease, supporting epidemiologic observations of fibrosis occurring in families.⁶⁰

Immunocompromised individuals with HIV shed fewer eggs than similar individuals without HIV.⁶¹ Secretions from schistosome eggs, termed soluble egg antigens (SEAs), trigger host inflammatory and immune responses that result in granuloma formation, are T-cell-dependent and include prominent Th2 com-

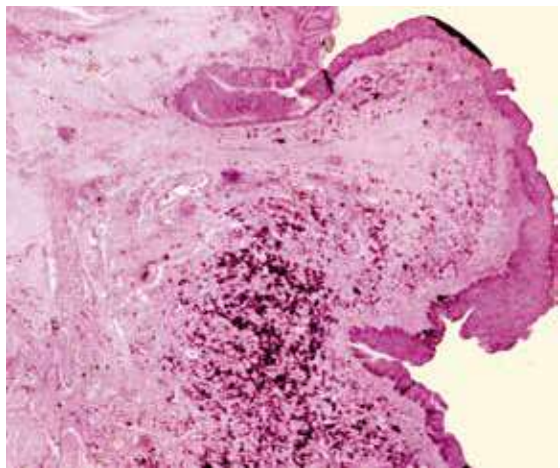


Figure 33.17. Histological section of bladder with pseudopolyp due to chronic infection with *S. haematobium*.



Figure 33.18. X-ray of bladder with a squamous cell tumor induced by *S. haematobium* eggs.

ponents.^{62, 63} Th2 bias downregulates other Th1 responses and alters host susceptibility patterns to other infectious pathogens, possibly including HIV.^{64, 65} In a study from Uganda, HIV and *S. mansoni* coinfection was associated with altered anti-HIV responses, notably lower frequency of bifunctional (IFN γ + IL-2 - TNF- α +) CD4 T cells and reduced HIV ADCC antibody titers, when compared to participants with HIV but not infected with *S. mansoni*.⁶⁶

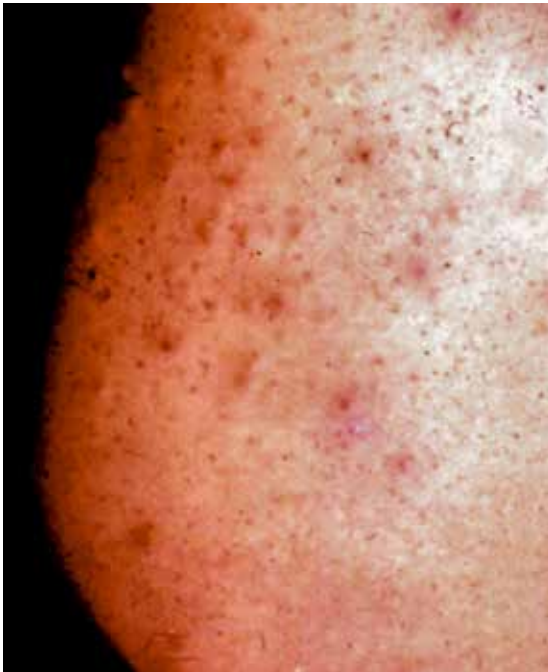


Figure 33.19. Thigh of a child suffering from a maculopapular rash ("swimmer's itch") due to the cercariae of a schistosome species that normally infects birds.

The pathogenesis of granuloma formation in a murine model requires the production of TNF- α .⁶⁷ The granulomas' diameters vary with the age of the infection; the longer the infection persists, the smaller they are in diameter. Type 2 cytokines (e.g., IL-4, IL-5, IL-13) accumulate in granulomas together with other multi-cellular components, including macrophages, eosinophils, and mast cells. In newly acquired infections, granulomas are large, displacing normal tissue with fibrotic, epithelioid reactions. Over time, eggs elicit less volume of granulomatous tissue, with IL-13 acting to influence fibrosis via alternatively activated macrophages and fibroblasts.^{68, 69} TNF- α does not affect worm fecundity, but it alters the timing and rate at which eggs are produced in vitro, which could affect disease progression.⁷⁰

Granulomas form around eggs that collect in the intestinal wall and result in fibrosis. Erosion of the submucosa and villous

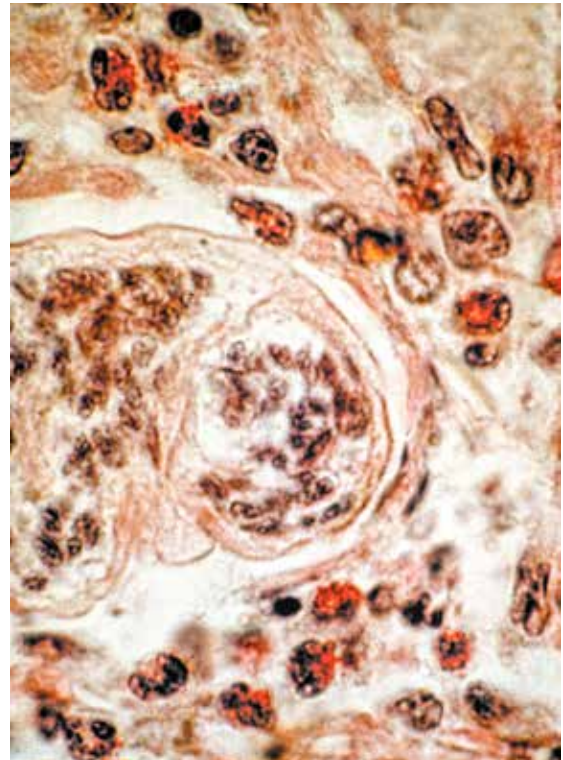


Figure 33.20. Cercaria of *S. mansoni* in skin surrounded by eosinophils.

tissue also occurs, presumably by the action of secreted proteolytic enzymes from the miracidia within the eggs. In heavy infection, gastrointestinal (GI) hemorrhage results from damage to the submucosa. An emerging appreciation for the gut microbiome and, more specifically, gut dysbiosis and the relationship between infection and clinical disease is currently an active area of research.⁷¹⁻⁷³

Eggs swept back into the liver block pre-sinusoidal capillaries and induce granulomas there. The presence of granulomas causes tissue fibrosis and eventually leads to obstruction of the hepatic vasculature. Fibrosis of most portal areas incorporating the blood vessels leads to pipe stem fibrosis (Symmers' fibrosis) (Fig. 33.15) and, ultimately, portal hypertension. Clinically, this manifests as hepatosplenomegaly, the extent of which is dependent partially on host major histocompatibility class II alleles.⁷⁴ Development of collateral circulation follows, including esophageal varices. Parenchymal liver cells remain unaffected by granulomas, and liver functions remain normal.

Portal hypertension results in eggs bypassing the liver instead of carrying many of them to the spleen, which then enlarges, increasing pressure even more in the portal circulation. Infection with *S. japonicum* results in more granulomas and morbidity than with *S. mansoni* because it produces five to ten times more eggs. Collateral circulation also transports eggs to the capillary beds of the lungs. This sequela frequently leads to pulmonary fibrosis and cor pulmonale in heavy, long-term infections.

Accumulation of *S. haematobium* eggs around the bladder and ureters induces granuloma formation and fibrosis. Calcification of dead eggs (Fig. 33.16) results in bladder rigidity and subsequent increased hydrostatic pressure in the ureters and kidneys. The blad-

der epithelium develops pseudopolyps (Fig. 33.17), which can transform into transitional squamous cell carcinoma in untreated patients (Fig. 33.18), which is often fatal.

In some patients with long-standing disease caused by any species of *Schistosoma*, the deposition of immune complexes in the kidneys can lead to basement membrane disease.⁷⁵

Over the last decade, clinical evidence confirms that *S. haematobium* eggs also gain access to the female genital tract, leading to FGS. Granulomas in the uterus, cervix, and vagina produce ulcerative lesions, which are rich in inflammatory cells. These lesions bleed easily and presumably provide conduits for the entry of HIV during intercourse.¹⁴ *S. haematobium* is responsible for a 3–4-fold increase in acquiring HIV/AIDS.

Penetration of the skin by cercariae depends on the worm releasing proteases and eicosanoids. The process of host entry typically occurs asymptotically. Still, repeated exposure can lead to sensitization and a maculopapular rash (Fig. 33.19), characterized by IgE or IgG antibodies and an eosinophilic infiltrate. This is particularly true of skin penetration by avian or bovine schistosomes that remain localized in the skin and cannot mature to adults in humans. Avian schistosomes of the genera *Austroilharzia*, *Trichobilharzia*, and *Ornithobilharzia*, and other mammalian schistosomes (*S. matthei* and *Schistosomatium douthitti*) are included in this group. The cercariae of these species cause a hypersensitivity skin reaction (cercarial dermatitis), known as “clam digger’s itch” or “swimmer’s itch” (Fig. 33.19).

Cellular and humoral responses to penetrating cercariae and migrating schistosomulae are critical components of naturally acquired immunity to human schistosomiasis. This

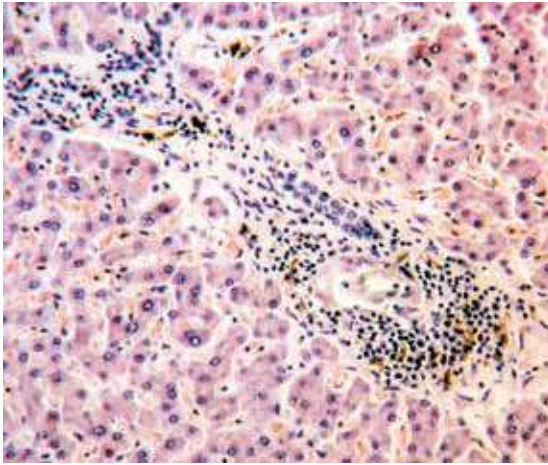


Figure 33.21. Granuloma surrounding an egg of *S. mansoni* in liver tissue.

derives from experimental evidence that cercariae, attenuated by exposure to ionizing radiation (e.g., x-rays, gamma-rays, or UV), can penetrate skin and migrate through the tissues without transforming into schistosomulae. Attenuated cercariae elicit protective immune responses, including interleukin-13.⁷⁶⁻⁷⁸ These observations are the basis for an experimental vaccine in non-human primates. The cercariae must remain alive to secrete the antigens associated with vaccine protection.

Protective immunity may take years of exposure to viable cercariae for those living in endemic regions. Children have a particular problem mounting an effective immune response to invading schistosomulae. Young children are highly susceptible to the parasite. But as they grow older, most of them become resistant to re-infection. The precise mechanism responsible for this change in susceptibility is likely due to age-related aspects of the development of the innate immune response.^{51, 79} Another plausible hypothesis suggests that young children respond initially to the parasite by producing IgG₁-blocking antibodies.⁸⁰

Current understanding of Th1 and Th2 immune mechanisms helps inform the selec-

tion of recombinant schistosome protein antigens for use in producing a practical vaccine. A controlled human infection model using male cercaria unable to produce eggs after developing into adult worms serves as a good model for future vaccine studies and understanding the changes in cytokine patterns and cellular and humoral immune responses after infection.⁸³

Clinical Disease

As in other helminth infections, clinical disAs with other helminth infections, serious illness usually occurs only in heavily infected individuals. A frequent clinical symptom of acute schistosomiasis occurs predominantly with infection caused by *S. japonicum* or *S. mansoni*. It is sometimes referred to as “Katayama fever.” Chronic infection with *S. haematobium* can lead to squamous cell carcinoma of the bladder.

Acute schistosomiasis (Katayama fever)

The dramatic clinical manifestations of Katayama fever occur most commonly in new immigrants who experience intense exposure to either *S. japonicum* or *S. mansoni* cercariae. The name reflects the early descriptions of this syndrome in the Katayama District of Japan.⁸⁴

The symptoms are often dramatic and appear approximately 4–8 weeks after initial exposure when adult female worms release eggs into the tissues. Katayama fever can resemble some of the manifestations of serum sickness and has a clinical resemblance to typhoid fever. Patients experience hepatosplenomegaly and lymphadenopathy, as well as impressive eosinophilia. The affected individual is frequently febrile and has flu-like symptoms, including cough and headache. At this stage of the illness, schistosome eggs may not yet have appeared in the feces or urine.

Chronic schistosomiasis

This manifestation of infection occurs due to many years of progressive injury resulting from chronic egg deposition in the tissues and the resulting granuloma formation (Fig. 33.21). The injury has an immunopathological basis. In the case of *S. japonicum* and *S. mansoni* infection, the injury occurs when eggs are deposited in the wall of the intestine and the liver parenchyma. With *S. haematobium*, injury occurs in the bladder.

The extent of injury depends on the number of worm pairs present, so chronic schistosomiasis occurs predominantly in individuals predisposed to develop heavy infections.⁸⁰ In a population with repeated high exposures to cercariae, less than a quarter acquire a heavy infection, and only 10% of these later individuals develop periportal fibrosis.

S. japonicum and *S. mansoni* infections result in chronic intestinal and hepatic dysfunction. Children with intestinal schistosomiasis develop intermittent abdominal pain, sometimes accompanied by bloody diarrhea. The blood loss and ulceration of intestinal schistosomiasis may result in iron deficiency and anemia. This may explain why chronic schistosomiasis during childhood can result in physical growth retardation similar to that described for intestinal nematode infections. Stunting becomes most prominent at the age of peak intensity (usually 8–20 years of age).⁸⁵ It is partly reversible by specific anthelmintic therapy.⁸⁶

Hepatomegaly results from portal fibrosis. Splenomegaly follows, and in advanced cases, the spleen may fill much of the left side of the abdomen. Patients may also develop symptoms of hypersplenism. Portal obstructive disease due to schistosomiasis is similar to other causes in that it leads to hematemesis from ruptured esophageal varices. As a result of portal hypertension and the consequent

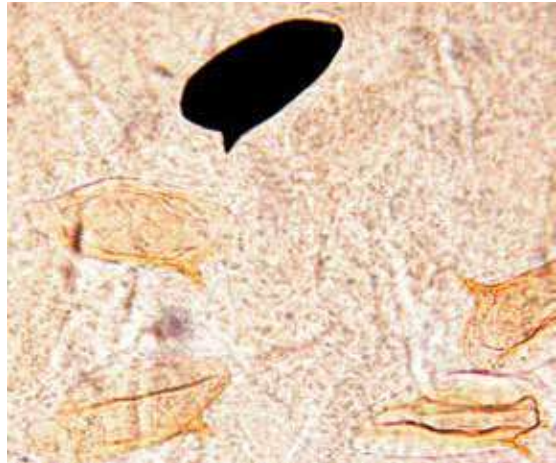


Figure 33.22. Biopsy of rectal tissue revealing eggs of *S. mansoni*. Note calcified egg, indicating that the infection was chronic.

development of collateral circulation, schistosome eggs are washed into the lungs, where they induce granulomatous inflammation, leading to obstructive disease culminating in *cor pulmonale*. As noted above, prolonged infections can cause nephrotic syndrome, resulting from the deposition of immune complexes onto the glomerular membrane.

S. haematobium causes involvement of the urinary tract, which is characterized by an inflammatory response induced by the secretions of the miracidia inside the eggs as they are deposited in the wall of the bladder. Patients with chronic *S. haematobium* infection develop hematuria and symptoms that mimic urinary tract infections such as dysuria and increased urinary frequency. Over time, the inflammatory changes in the bladder may result in fibrosis that can lead to obstructive uropathy. This sometimes results in hydronephrosis or hydroureter. The resulting urinary stasis can sometimes lead to secondary bacterial urinary tract infections that may exacerbate the scarring and fibrosis.

Bladder carcinoma

A characteristic type of bladder carcinoma occurs in many regions where *S. haematobium* is endemic. In contrast to adeno-

carcinoma, the most common type of bladder cancer in industrialized countries, some patients with chronic *S. haematobium* go on to develop squamous cell carcinoma. Evidence suggests that the eggs of *S. haematobium* can induce this through several mechanisms, including the action of estrogen metabolites.⁸⁷ Squamous cell carcinoma is the most common type of bladder cancer in parts of Egypt, as well as elsewhere in Africa. Over time, *S. haematobium* eggs may function as a human co-carcinogen that elicits metaplastic changes in the bladder.⁸⁸ Given the heterogeneity and variable bladder cancer risk in geographies endemic to *S. haematobium* infection, this association requires further molecular and genomic studies.⁸⁹

Female genital schistosomiasis (FGS)

Egg deposition in the uterus, cervix, and lower genital tract produces a painful and stigmatizing condition known as FGS. FGS is associated with bleeding, vaginal itching, and pain during sexual intercourse. On colposcopic exam, FGS presents as “sandy patches” corresponding to schistosome granulomas. These patches bleed easily on contact. FGS also has significant psychosocial consequences and has been linked to stigma, marital discord, and depression. There is also a strong association between FGS and acquiring HIV during sex, with some estimates indicating that FGS is a significant co-factor in Africa’s AIDS epidemic.¹⁴ Male genital schistosomiasis, although less well studied, nevertheless causes considerable morbidity, with reporting most often from areas endemic for *S. haematobium* and in men without access to praziquantel.⁹⁰

CNS schistosomiasis

Schistosomes rarely induce focal inflammatory reactions within the central nervous system (CNS), caused by the deposition of eggs in the spinal cord and the brain.⁹¹ *S. mansoni* and *S. haematobium* are more likely

to do so in the spinal cord, and *S. japonicum* in the brain. Inflammation due to eggs may result in focal transverse myelitis and encephalopathy.

Diagnosis (see Clinical Appendix)

Diagnosing schistosomiasis can be done by detecting the parasite or probing the host’s immune response. Definitive diagnosis is made by microscopically identifying schistosome eggs in stool or urine (Figs. 33.4, 33.5a, 33.5b, 33.6). If a single stool examination is negative, the concentration of a specimen collected over 24 hours is required because the number of eggs in stool can vary greatly, even over short periods. Quantitative egg counts are sometimes helpful for epidemiologic studies attempting to determine infection intensities.

A rectal biopsy can be carried out for light infections or in patients whose egg excretion is intermittent and whose eggs cannot be found in stool (Fig. 33.22). The tissue is squashed between two microscope slides and examined under the low-power lens of a microscope. If eggs are detected, they can be observed under higher power and examined for “flame” cells (excretory cells). If they are flickering (as in a flame), then the miracidium in the egg is alive, and the patient has an active infection. Treatment must then be entertained. If no live eggs are seen, or if they are calcified, then it is likely that the infection is no longer active, and treatment is not necessary. It is helpful to refer to the specimen as a “rectal snip” rather than a biopsy to preclude its fixation and subsequent sectioning, making the identification of live miracidia in eggs impossible.

While most schistosome eggs appear in feces, urine should be examined for eggs of *S. haematobium* if this species is suspected. The urine sample should be collected when

egg excretion is maximal at noon. Urine may have to be concentrated by sedimentation to reveal the few eggs present. *S. haematobium* eggs may also be seen in stool and rectal snip specimens, but their numbers are typically small in these samples. There are now also polymerase chain reaction (PCR) techniques that offer advantages over microscopic diagnoses.⁹²

Diagnosing FGS requires training to identify the characteristic sandy patches associated with this condition. Confirmatory microscopy is a valuable aid, but it is only sometimes available in resource-poor settings.

Several additional tests have become available for the diagnosis of schistosomiasis. Two schistosome glycoprotein antigens, CCA and CAA that circulate in the bloodstream of acutely infected patients have been identified and can now be tested using qualitative and quantitative assays.⁹³⁻⁹⁵ These assays can be easily applied to urine samples.^{96, 97} Several NAATs have been developed to detect and aid in schistosome speciation.⁹⁸⁻¹⁰⁰ Some studies find that the CCA assay exhibits high sensitivity for *S. mansoni* infection (although moderate specificity), whereas PCR-based assays are preferred for *S. haematobium* infection.⁹²

Antibodies develop 6–12 weeks after exposure and tend to become positive before eggs are evident in urine or stool.¹⁰¹ Several serological tests are also available, including western blot confirmatory testing.¹⁰² Since serologies remain positive for long periods after treatment, they cannot be used for follow-up. They are challenging to interpret when used to monitor repeat infections in endemic areas.¹⁰³

Portable ultrasound imaging is clinically helpful in diagnosing schistosomiasis. Ultrasound can define the extent of Sym-

mers' fibrosis in patients with *S. mansoni* or *S. japonicum* infections. At the same time, the chronic obstructive changes associated with *S. haematobium* infection can also be detected.¹⁰⁴

Treatment (see Clinical Appendix)

Optimal treatment depends on whether one is treating acute schistosomiasis syndrome (Katayama fever) or the chronic phase of the disease. Since acute schistosomiasis syndrome is a hypersensitivity reaction to the parasitic antigens, anthelmintic therapy results in worsening of symptoms in about half of those treated.¹⁰⁵ Short-course treatment with corticosteroids and delay of anthelmintic therapy improves symptoms, leads to decreased morbidity, and avoids the clinical deterioration seen with acute use of anthelmintic treatment.^{105, 106}

Acute treatment with anthelmintic therapy is not required to prevent the chronic manifestations of schistosomiasis.¹⁰⁷ How long to wait after the acute syndrome subsides before initiating anthelmintic therapy is problematic. It is prudent to wait for a period (6 weeks according to some) after resolution of the acute symptoms to start treatment when the worms have fully matured, as praziquantel, the drug of choice for schistosomiasis is not active against the larval stage.¹⁰⁷ Treatment can be repeated 4–6 weeks after this first treatment course.

For treating chronic schistosomiasis, praziquantel is the drug of choice for most species of schistosomes. This drug is well-tolerated, is associated with few side effects (nausea, epigastric pain, dizziness, and general malaise), has a very high therapeutic index, and a single dose is usually sufficient to significantly reduce the worm burden in individuals in endemic areas with



Figure 33.23. Lake Nasser and the Aswan High Dam in Egypt. Photo S. Musgrave, astronaut *extraordinaire*.

high worm burdens and to cure those with low worm burdens.¹⁰⁸⁻¹¹⁰ Praziquantel interferes with calcium ion influx across the tegument, resulting in spastic paralysis of the worm. At higher doses, the tegument develops blebs and is unmasked, making it susceptible to immune attack.¹¹¹ In younger patients, praziquantel may reverse some pathology associated with Symmers' fibrosis.¹¹² Evidence indicates that part of its effectiveness is synergistic with the host's humoral immune response.¹¹³ Because it is effective in a single dose, it has been used in control programs (see below).

For patients no longer in endemic areas, the test of cure is recommended with repeat testing for eggs in urine or stool no sooner than 3–6 months after treatment.¹¹⁴ Patients should be monitored for evidence of CNS disease when treatment is initiated, as treatment can cause an acute inflammatory

response in patients with eggs in the CNS. If this develops, prompt and prolonged therapy with corticosteroids is critical to prevent the worsening of neurological symptoms and irreversible damage.^{115, 116}

Praziquantel is now inexpensive, and the World Health Assembly has endorsed community treatment of school-age children in endemic areas. Unlimited Health (formerly the Schistosomiasis Control Initiative) in London is leading global efforts to provide mass drug administration of praziquantel. Currently, praziquantel is available in “rapid impact” interventions, including mass-drug treatments for intestinal helminth infections, lymphatic filariasis, and onchocerciasis.¹¹⁷ Linking this approach with antiretroviral drug therapy for HIV/AIDS, available through established access programs from The Global Fund and PEPFAR, would allow for a holistic approach to health care for millions of girls and women living in low-income countries.¹¹⁸ Resistance to praziquantel has occurred, and treatment failures have been reported.¹¹⁹⁻¹²¹

Alternatives to praziquantel are limited in use due to a higher frequency of adverse reactions and differences in the spectrum of activity. Oxamniquine is an alternate drug with good anti-parasitic activity. In some regions, oxamniquine is as effective as praziquantel for treating infections with *S. mansoni*, and metrifonate is effective for treating *S. haematobium* infections.¹²² The anti-malarial drug artemether has been studied in China as a chemoprophylactic agent in those anticipating high exposure to *S. japonicum* and *S. mansoni* cercariae during seasonal floods. The chemoprophylactic activity of artemether was present but lower against *S. haematobium*.¹²³ The efficacy of praziquantel is enhanced when combined

with artemether, and the combination might prevent the emergence of resistance to praziquantel when used in widespread and repeated community treatment.¹²⁴ A recent trial for *S. haematobium* infection found that the antimalarial drug artesunate-mefloquine was non-inferior to praziquantel,¹²⁵ which may become an important observation in areas where malaria and schistosomiasis are co-endemic. Similarly, when co-administered with dihydroartemisinin-piperaquine, praziquantel may be more effective for *S. mansoni* infection than praziquantel alone.¹²⁶

Treatment should be carried out only in patients with active schistosome infections. Portocaval or splenorenal shunts should be avoided in untreated schistosomiasis because they increase the probability of eggs reaching the lungs. If the intensity of portal hypertension mandates such a shunt, it should be carried out only after treating with any of the drugs mentioned above.

Prevention and Control

Schistosomes' success in carrying out their life cycles depends upon complex ecological interactions with various invertebrate and vertebrate host species. They appear to have many weak points in their quest to complete their life cycles. Numerous control programs have attempted to take advantage of these vulnerabilities. Control programs in the Middle East and North Africa have nearly succeeded in schistosome elimination, while programs in China and Brazil have also achieved remarkable success.¹²⁷⁻¹³⁰ Although more challenging for less-developed countries, several programs, including mass treatment to reduce worm burden, have improved the situation for many.^{131, 132}

Individuals' prevention of schistosomiasis requires that they never come in contact with contaminated freshwater. This suggestion is impossible to carry out in much of the world because of many complex economic, cultural, and behavioral patterns. In addition, many people may need to be in contact with fresh water for agricultural or other food-gathering purposes. Temporary visitors to endemic areas can heed the advice to avoid potential sources of infection. Dam building in Africa has facilitated the spread of schistosomiasis (Fig. 33.23).

The control of schistosomiasis at the community level has been directed at 1. eradication of snail intermediate hosts with molluscicides and biologic agents, 2. public health education, 3. sanitation or other engineering interventions concerning freshwater supplies, and 4. chemotherapy with praziquantel and oxamniquine.^{133, 134}

Control of *S. japonicum* is complicated by reservoir hosts, such as water buffalo, non-human primates, and cattle, in many regions of Asia. This problem was overcome in Japan mainly by eliminating water buffalo in rice farming and using horses (a non-susceptible host) as draft animals instead.¹⁸

The gold standard of control for schistosomiasis has been the mass-drug administration of praziquantel, with the London-based Unlimited Health, together with other non-governmental development organizations, leading the way to provide tens of millions of people access to this essential medicine. Support for such programs of mass-drug administration comes through overseas development agencies such as USAID and some private donations, including the innovative New York-based END Fund. The WHO NTD Roadmap contains comprehensive mitigation steps and recommendations for measuring the resulting outcomes

toward eliminating schistosomiasis. The interventions include broad efforts such as monitoring mass drug administration, snail control, proper sanitation, and monitoring reintroduction of schistosomiasis via migration of infected individuals into regions where the disease was eliminated by mass drug administration and ancillary efforts. In addition, an argument has been made for more resources focused on disease prevalence monitoring and elimination efforts beyond just high-intensity infection areas.¹³⁵

Studies in endemic areas have shown that while praziquantel is effective at treating large populations, there is a high rate of post-treatment reinfection. This necessitates frequent administration of the drug, although this tactic is often only possible in poor, developing rural areas with the support of the international community.¹³⁶ Control of the infection with anthelmintic drugs alone is challenging due to a wide variety of factors in addition to recurrent infections. The St Lucia project (1965-1981) employed environmental, snail control, and targeted drug control measures that could inform future integrated control strategies in areas struggling with disease elimination, mainly based on mass drug treatment.¹³⁷ There is concern regarding reliance on a single drug for mass administration. The emergence of praziquantel drug resistance has been documented.¹³⁸ This highlights the urgent need for continued investigations into vaccinations and new therapeutics to facilitate the elimination efforts for this neglected tropical disease.^{51, 75, 81, 139-147} New drugs with activ-

ity against the juvenile cercarial form of the schistosome lifecycle would aid overall control efforts and complement the use of praziquantel in treating adult worms. A call for adequate resources and considerations for new drug development was made at a 2022 workshop highlighting current challenges.¹⁴⁸

For new vaccines, animal protection studies have used several naturally purified or recombinant proteins such as para myosin, calpain, tetraspanin, and others with good results in a mouse model. However, the mechanism of protection is still under investigation.^{142, 147} Studies in the Philippines in a population with a risk of exposure to *S. japonicum* demonstrated that individuals with predominantly Th1 cellular immune responses appeared resistant to initial infection.^{149, 150} Several different vaccine constructs have entered human clinical trials. Unfortunately, low efficacy was reported from a Phase 3 trial of the most advanced trial.¹⁵¹ However, three next-generation recombinant protein vaccines using a synthetic lipid A adjuvant are undergoing phase 1-2 clinical trials in Brazil or Africa; they include an Sm-TSP-2 tetraspanin, a Sm14 antigen, and a Sm-p80 calpain.^{152, 153} These recombinant protein vaccines could accelerate the elimination of schistosomiasis faster than mass drug therapy alone. A better functional characterization of the proteins encoded by the already sequenced schistosome genomes will hopefully lead to experimental vaccine constructs with a higher probability of success in human testing.¹⁵⁴

References

1. Gurarie, D., et al., Modeling the effect of chronic schistosomiasis on childhood development and the potential for catch-up growth with different drug treatment strategies promoted for control of endemic schistosomiasis. *Am J Trop Med Hyg* **2011**, *84* (5), 773-81.
2. Organization, W. H., Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva. Licence: CC BY-NC-SA 3.0 IGO **2020**.

3. IHME, Schistosomiasis — Level 3 cause. https://www.healthdata.org/results/gbd_summaries/2019/schistosomiasis-level-3-cause accessed April 30, 2014 **2024**.
4. King, C. H., Parasites and poverty: the case of schistosomiasis. *Acta Trop* **2010**, *113* (2), 95-104.
5. Lammie, P. J., et al., Eliminating lymphatic filariasis, onchocerciasis, and schistosomiasis from the americas: breaking a historical legacy of slavery. *PLoS Negl Trop Dis* **2007**, *1* (2), e71.
6. Hewitt, R.; Willingham, A. L., Status of Schistosomiasis Elimination in the Caribbean Region. *Trop Med Infect Dis* **2019**, *4* (1).
7. Fuller, G. K., et al., Schistosomiasis in Omo National Park of southwest Ethiopia. *Am J Trop Med Hyg* **1979**, *28* (3), 526-30.
8. Douard, A., et al., Urinary schistosomiasis in France. *Int J Infect Dis* **2011**, *15* (7), e506-7.
9. Berry, A., et al., *Schistosomiasis haematobium*, Corsica, France. *Emerg Infect Dis* **2014**, *20* (9), 1595-7.
10. Wellinghausen, N., et al., A family cluster of schistosomiasis acquired in Solenzara River, Corsica (France) - Solenzara River is clearly a transmission site for schistosomiasis in Corsica. *Parasitol Res* **2022**, *121* (8), 2449-2452.
11. Onyekwere, A. M., et al., Population Genetic Structure and Hybridization of *Schistosoma haematobium* in Nigeria. *Pathogens* **2022**, *11* (4).
12. Diaz, A. V., et al., Reaching the World Health Organization elimination targets for schistosomiasis: the importance of a One Health perspective. *Philos Trans R Soc Lond B Biol Sci* **2023**, *378* (1887), 20220274.
13. Hotez, P. J., et al., Female Genital Schistosomiasis. *N Engl J Med* **2019**, *381* (26), 2493-2495.
14. Kjetland, E. F., et al., Genital schistosomiasis and its unacknowledged role on HIV transmission in the STD intervention studies. *Int J STD AIDS* **2014**, *25* (10), 705-15.
15. Chu, T., et al., Prevalence of *Schistosoma intercalatum* and *S. haematobium* Infection among Primary Schoolchildren in Capital Areas of Democratic Republic Of Sao Tome and Principe, West Africa. *Iran J Parasitol* **2012**, *7* (1), 67-72.
16. Dufillot, D., et al., [Prevalence of *Schistosoma intercalatum* intestinal bilharziasis in children hospitalized the urban area of Gabon]. *Arch Pediatr* **1995**, *2* (10), 1023-4.
17. World Health Organization. *Weekly Epidemiol Rec* **171** **1989**, *64*.
18. Minai, M., et al., Historical view of Schistosomiasis japonica in Japan: implementation and evaluation of disease-control strategies in Yamanashi Prefecture. *Parasitology international* **2003**, *52* (4), 321-6.
19. Gordon, C. A., et al., Schistosomiasis in the People's Republic of China - down but not out. *Parasitology* **2022**, *149* (2), 218-233.
20. Hotez, P. J., et al., Emerging and reemerging helminthiases and the public health of China. *Emerging infectious diseases* **1997**, *3* (3), 303-10.
21. Kongklieng, A., et al., Molecular differentiation of *Schistosoma japonicum* and *Schistosoma mekongi* by real-time PCR with high resolution melting analysis. *Korean J Parasitol* **2013**, *51* (6), 651-6.
22. Lawton, S. P., et al., Genomes and geography: genomic insights into the evolution and phylogeography of the genus *Schistosoma*. *Parasit Vectors* **2011**, *4*, 131.
23. Ruffer, M. A., Note on the Presence of “*Bilharzia Haematobia*” in Egyptian Mummies of the Twentieth Dynasty [1250-1000 B.C.]. *Br Med J* **1910**, *1* (2557), 16.
24. Nunn, J. F.; Tapp, E., Tropical diseases in ancient Egypt. *Trans R Soc Trop Med Hyg* **2000**, *94* (2), 147-53.
25. Renoult, A. J., Notice sur l'he'maturie qu'e'prouvent les Europe'ens dans la haute Egypte et la Nubie. *J. Gen. Med. Chir. Pharm* **1808**, *17*, 366-370.
26. Cox, F. E., History of human parasitology. *Clin Microbiol Rev* **2002**, *15* (4), 595-612.
27. Bilharz, T.; von Siebold, C. T., Ein Beitrag zur Helminthographia humana, aus brieflichen Mitteilungen des Dr. Bilharz in Cairo, nenst Bermerkungen von Prof. C. Th. von Siebold in Breslau. *Z. Wiss. Zool.* **1852**, *4*, 53-76.
28. Bilharz, T., Fernere mittheilungen u'ber Distomun haematobium. *Z. Wiss. Zool.* **1853**, *4*, 454-456.

29. Kean, B. H., et al., Tropical medicine and parasitology: classic investigations. *Cornell University Press, Ithaca, NY* **1978**.
30. Griesinger, W., Klinische und anatomische Beobachtungen uber die Krankheiten von Aegypten. *Arch Physiol Heilk* **1854**, *13*, 528-575.
31. Leiper, R. T., Researches on Egyptian Bilharziosis. 1918.
32. Manson, P., Report of a Case of Bilharzia from the West Indies. *British medical journal* **1902**, *2* (2190), 1894-5.
33. Sambon, L. W.; J., New or little known African entozoa. *Med Hyg* **117** **1907**, *10*.
34. Piraja de Silva, M. A., Contribucao para o estudo da schistosomiasena Bahia. *Brazil Med* **1908**, *2* 281-283.
35. Majima, T., A strange case of liver cirrhosis caused by parasitic ova. *Tokyo Igakkai Zasshi* **1888**, *2*, 898-901.
36. Katsurada, F., The etiology of a parasitic disease. *Iji Shimibun* **1904**, *669*, 1325-1332.
37. Catto, J., *Schistosoma cattoi*, a new blood fluke of man. *British medical journal* **1905**, *1* (2297), 11-26.2.
38. Kawanishi, K., A report on a study of the "Katayama disease" in Higo-No-Kuni. *Tokyo Ig Za* **1904**, *18*, 31-48.
39. Fujinami, K.; Nakamura, H., Katayama disease in Hiroshima prefecture: route of infection, development of the worm in the host and animals in Katayama disease in Hiroshima prefecture (Japanese blood sucking worm disease-schistosomiasis japonica). . *Kyoto Ig Za* **1909**, *6*, 224-252.
40. Miyagawa, Y., Uber den Wanderungsweg des Schistosomum japonicum von der Haut bis zum Pfortadersystem und uber die Korperkonstitution der jungsten Wurmer zur Zeit der Hautinvasion. *Zentralbl Bakteriол Parasit Infekt* **1912**, *66*, 406-417.
41. Miyairi, K.; Suzuki, M., Der Zwischenwirt der Schistosoma japonicum Katsurada. *Mitt Med Fakultat Kaiserlichen Univ Kyushu* **1914**, *1* 187-197.
42. Kierman, F. A., The blood fluke that saved Formosa. *Harper Magazine* **1959**, 45-47.
43. Hotez, P., Forgotten People, Forgotten Diseases: The Neglected Tropical Diseases and their Impact on Global Health and Development. **2013**.
44. Foster, W. D., A history of parasitology. *Livingstone, Edinburgh, United Kingdom*. **1965**.
45. Wilson, R. A., The saga of schistosome migration and attrition. *Parasitology* **2009**, *136* (12), 1581-92.
46. Vermund, S. H., et al., Survival of *Schistosoma mansoni* in the human host: estimates from a community-based prospective study in Puerto Rico. *The American journal of tropical medicine and hygiene* **1983**, *32* (5), 1040-8.
47. You, H., et al., Revisiting glucose uptake and metabolism in schistosomes: new molecular insights for improved schistosomiasis therapies. *Front Genet* **2014**, *5*, 176.
48. Chappell, C. L., et al., The hypersensitivity response to the adult worm proteinase, SMw32, in *Schistosoma mansoni* infected mice. *Am J Trop Med Hyg* **1988**, *39* (5), 463-8.
49. Kruger, F. J.; Hamilton-Attwell, V. L., The morphology of a sensory receptor in the nipped tubercles of *Schistosoma mattheei*. *Onderstepoort J Vet Res* **1985**, *52* (2), 111-2.
50. Chen, R., et al., A male-derived nonribosomal peptide pheromone controls female schistosome development. *Cell* **2022**, *185* (9), 1506-1520 e17.
51. McManus, D. P.; Loukas, A., Current status of vaccines for schistosomiasis. *Clin Microbiol Rev* **2008**, *21* (1), 225-42.
52. Ruppel, A., et al., Invasion by schistosome cercariae: neglected aspects in *Schistosoma japonicum*. *Trends in parasitology* **2004**, *20* (9), 397-400.
53. Cohen, F. E., et al., Arresting tissue invasion of a parasite by protease inhibitors chosen with the aid of computer modeling. *Biochemistry* **1991**, *30* (47), 11221-9.
54. Jenkins, S. J., et al., Modulation of the host's immune response by schistosome larvae. *Parasite Immunol* **2005**, *27* (10-11), 385-93.
55. Cai, P., et al., The Tao survivorship of schistosomes: implications for schistosomiasis control. *Int J Parasitol* **2016**.
56. Collins, J. J., et al., Stem cell progeny contribute to the schistosome host-parasite interface. *Elife*

- 2016, 5.
57. Gendrel, D., et al., Nontyphoidal salmonella septicemia in Gabonese children infected with *Schistosoma intercalatum*. *Clin Inf Dis* **1994**, 18 (1), 103-5.
 58. Webster, J. P., et al., Do hosts and parasites coevolve? Empirical support from the *Schistosoma* system. *Am Nat* **2004**, 164 Suppl 5, S33-51.
 59. Chiarella, J. M., et al., Absence of linkage between MHC and a gene involved in susceptibility to human schistosomiasis. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica ... [et al.]* **1998**, 31 (5), 665-70.
 60. Dessein, A. J., et al., Severe hepatic fibrosis in *Schistosoma mansoni* infection is controlled by a major locus that is closely linked to the interferon-gamma receptor gene. *American journal of human genetics* **1999**, 65 (3), 709-21.
 61. Karanja, D. M., et al., Studies on schistosomiasis in western Kenya: II. Efficacy of praziquantel for treatment of schistosomiasis in persons coinfectd with human immunodeficiency virus-1. *Am J Trop Med Hyg* **1998**, 59 (2), 307-11.
 62. Warren, K. S.; Ser, A., The pathology of schistosome infections. *Helminth Abstr* **1973**, 42 591-633.
 63. King, C. L., et al., Mice with a targeted deletion of the IgE gene have increased worm burdens and reduced granulomatous inflammation following primary infection with *Schistosoma mansoni*. *Journal of immunology (Baltimore, Md. : 1950)* **1997**, 158 (1), 294-300.
 64. Pearce, E. J., et al., Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, *Schistosoma mansoni*. *The Journal of experimental medicine* **1991**, 173 (1), 159-66.
 65. Curry, A. J., et al., Evidence that cytokine-mediated immune interactions induced by *Schistosoma mansoni* alter disease outcome in mice concurrently infected with *Trichuris muris*. *The Journal of experimental medicine* **1995**, 181 (2), 769-74.
 66. Obuku, A. E., et al., HIV specific Th1 responses are altered in Ugandans with HIV and *Schistosoma mansoni* coinfection. *BMC Immunol* **2023**, 24 (1), 25.
 67. Haseeb, M. A., et al., Elevated serum levels of TNF-alpha, sTNF-RI and sTNF-RII in murine schistosomiasis correlate with schistosome oviposition and circumoval granuloma formation. *Cytokine* **2001**, 15 (5), 266-9.
 68. Costain, A. H., et al., Corrigendum: Schistosome Egg Migration: Mechanisms, Pathogenesis and Host Immune Responses. *Frontiers in immunology* **2019**, 10, 749.
 69. Costain, A. H., et al., Schistosome Egg Migration: Mechanisms, Pathogenesis and Host Immune Responses. *Frontiers in immunology* **2018**, 9, 3042.
 70. Lopes-Junior, E. H., et al., Human tumor necrosis factor alpha affects the egg-laying dynamics and glucose metabolism of *Schistosoma mansoni* adult worms in vitro. *Parasit Vectors* **2022**, 15 (1), 176.
 71. Ajibola, O., et al., Urogenital schistosomiasis is associated with signatures of microbiome dysbiosis in Nigerian adolescents. *Sci Rep* **2019**, 9 (1), 829.
 72. Kay, G. L., et al., Differences in the Faecal Microbiome in *Schistosoma haematobium* Infected Children vs. Uninfected Children. *PLoS Negl Trop Dis* **2015**, 9 (6), e0003861.
 73. Staff, P. N. T. D., Correction: Differences in the Faecal Microbiome in *Schistosoma haematobium* Infected Children vs. Uninfected Children. *PLoS Negl Trop Dis* **2015**, 9 (7), e0003969.
 74. Secor, W. E., et al., Association of hepatosplenic schistosomiasis with HLA-DQB1*0201. *J Infect Dis* **1996**, 174 (5), 1131-5.
 75. Wynn, T. A., Development of an antipathology vaccine for schistosomiasis. *Annals of the New York Academy of Sciences* **1996**, 797, 191-5.
 76. Bickle, Q. D., et al., Resistance against *Schistosoma mansoni* induced by highly irradiated infections: studies on species specificity of immunization and attempts to transfer resistance. *Parasitology* **1985**, 90 (Pt 2), 301-12.
 77. Mangold, B. L.; Dean, D. A., The role of IgG antibodies from irradiated cercaria-immunized

- rabbits in the passive transfer of immunity to *Schistosoma mansoni*-infected mice. *Am J Trop Med Hyg* **1992**, 47 (6), 821-9.
78. Dessein, A., et al., Interleukin-13 in the skin and interferon-gamma in the liver are key players in immune protection in human schistosomiasis. *Immunological reviews* **2004**, 201, 180-90.
 79. Gryseels, B., et al., Human schistosomiasis. *Lancet* **2006**, 368 (9541), 1106-18.
 80. Acosta, L. P., et al., Antigen-specific antibody isotype patterns to schistosoma japonicum recombinant and native antigens in a defined population in Leyte, the Philippines. *Am J Trop Med Hyg* **2004**, 70 (5), 549-55.
 81. Siddiqui, A. A., et al., Schistosomiasis vaccines. *Hum Vaccin* **2011**, 7 (11), 1192-7.
 82. El Ridi, R., et al., Cysteine peptidases as schistosomiasis vaccines with inbuilt adjuvanticity. *PloS one* **2014**, 9 (1), e85401.
 83. Langenberg, M. C. C., et al., A controlled human *Schistosoma mansoni* infection model to advance novel drugs, vaccines and diagnostics. *Nat Med* **2020**, 26 (3), 326-332.
 84. Kajihara, N.; Hirayama, K., The War against a Regional Disease in Japan A History of the Eradication of *Schistosomiasis japonica*. *Trop Med Health* **2011**, 39 (1 Suppl 1), 3-44.
 85. McGarvey, S. T., et al., Child growth and schistosomiasis japonica in northeastern Leyte, the Philippines: cross-sectional results. *Am J Trop Med Hyg* **1992**, 46 (5), 571-81.
 86. Stephenson, L. S., et al., Single dose metrifonate or praziquantel treatment in Kenyan children. II. Effects on growth in relation to *Schistosoma haematobium* and hookworm egg counts. *Am J Trop Med Hyg* **1989**, 41 (4), 445-53.
 87. Santos, J., et al., Urinary estrogen metabolites and self-reported infertility in women infected with *Schistosoma haematobium*. *PloS one* **2014**, 9 (5), e96774.
 88. Hodder, S. L., et al., Predisposition to urinary tract epithelial metaplasia in *Schistosoma haematobium* infection. *Am J Trop Med Hyg* **2000**, 63 (3-4), 133-8.
 89. Jalloh, M., et al., Is Schistosomiasis a Risk Factor for Bladder Cancer? Evidence-Based Facts. *J Trop Med* **2020**, 2020, 8270810.
 90. Kayuni, S., et al., A systematic review with epidemiological update of male genital schistosomiasis (MGS): A call for integrated case management across the health system in sub-Saharan Africa. *Parasite Epidemiol Control* **2019**, 4, e00077.
 91. Scrimgeour, E. M.; Gajdusek, D. C., Involvement of the central nervous system in *Schistosoma mansoni* and *S. haematobium* infection. A review. *Brain : a journal of neurology* **1985**, 108 (Pt 4), 1023-38.
 92. Feleke, D. G., et al., Accuracy of Diagnostic Tests for Detecting *Schistosoma mansoni* and *S. haematobium* in Sub-Saharan Africa: A Systematic Review and Meta-Analysis. *Biomed Res Int* **2023**, 2023, 3769931.
 93. van Dam, G. J., et al., Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. *Journal of clinical microbiology* **2004**, 42 (12), 5458-61.
 94. van Dam, G. J., et al., A robust dry reagent lateral flow assay for diagnosis of active schistosomiasis by detection of *Schistosoma* circulating anodic antigen. *Exp Parasitol* **2013**, 135 (2), 274-82.
 95. Gundersen, S. G., et al., Early detection of circulating anodic antigen (CAA) in a case of acute schistosomiasis mansoni with Katayama fever. *Scand J Infect Dis* **1992**, 24 (4), 549-52.
 96. Coulibaly, J. T., et al., Accuracy of urine circulating cathodic antigen test for the diagnosis of *Schistosoma mansoni* in preschool-aged children before and after treatment. *PLoS Negl Trop Dis* **2013**, 7 (3), e2109.
 97. Coulibaly, J. T., et al., Accuracy of urine circulating cathodic antigen (CCA) test for *Schistosoma mansoni* diagnosis in different settings of Cote d'Ivoire. *PLoS Negl Trop Dis* **2011**, 5 (11), e1384.
 98. Schunk, M., et al., Use of occult blood detection cards for real-time PCR-based diagnosis of *Schistosoma Mansoni* Infection. *PloS one* **2015**, 10 (9), e0137730.
 99. Sady, H., et al., Detection of *Schistosoma mansoni* and *Schistosoma haematobium* by Real-Time PCR with High Resolution Melting Analysis. *Int J Mol Sci* **2015**, 16 (7), 16085-103.
 100. Meurs, L., et al., Is PCR the Next Reference Standard for the Diagnosis of *Schistosoma* in Stool? A Comparison with Microscopy in Senegal and Kenya. *PLoS Negl Trop Dis* **2015**, 9 (7),

- e0003959.
101. Jones, M. E., et al., Long seronegative window in *Schistosoma* infection. *Lancet* **1992**, 340 (8834-8835), 1549-50.
 102. Kinkel, H. F., et al., Evaluation of eight serological tests for diagnosis of imported schistosomiasis. *Clin Vaccine Immunol* **2012**, 19 (6), 948-53.
 103. Rabello, A. L., et al., Humoral immune responses in patients with acute *Schistosoma mansoni* infection who were followed up for two years after treatment. *Clin Infect Dis* **1997**, 24 (3), 304-8.
 104. Hatz, C., et al., Ultrasound in schistosomiasis--a critical look at methodological issues and potential applications. *Acta tropica* **1992**, 51 (1), 89-97.
 105. Jaureguiberry, S., et al., Acute schistosomiasis, a diagnostic and therapeutic challenge. *Clin Microbiol Infect* **2010**, 16 (3), 225-31.
 106. Harries, A. D.; Cook, G. C., Acute schistosomiasis (Katayama fever): clinical deterioration after chemotherapy. *J Infect* **1987**, 14 (2), 159-61.
 107. Grandiere-Perez, L., et al., Efficacy of praziquantel during the incubation and invasive phase of *Schistosoma haematobium* schistosomiasis in 18 travelers. *Am J Trop Med Hyg* **2006**, 74 (5), 814-8.
 108. Utzinger, J., et al., Efficacy of praziquantel against *Schistosoma mansoni* with particular consideration for intensity of infection. *Trop Med Int Health* **2000**, 5 (11), 771-8.
 109. Kramer, C. V., et al., Drugs for treating urinary schistosomiasis. *Cochrane Database Syst Rev* **2014**, 8, CD000053.
 110. Danso-Appiah, A., et al., Drugs for treating urinary schistosomiasis. *Cochrane Database Syst Rev* **2008**, (3), CD000053.
 111. Greenberg, R. M., Are Ca²⁺ channels targets of praziquantel action? *International journal for parasitology* **2005**, 35 (1), 1-9.
 112. Homeida, M. A., et al., Association of the therapeutic activity of praziquantel with the reversal of Symmers' fibrosis induced by *Schistosoma mansoni*. *Am J Trop Med Hyg* **1991**, 45 (3), 360-5.
 113. Brindley, P. J.; Sher, A., The chemotherapeutic effect of praziquantel against *Schistosoma mansoni* is dependent on host antibody response. *Journal of immunology (Baltimore, Md. : 1950)* **1987**, 139 (1), 215-20.
 114. Lucey, D. R.; Maguire, J. H., Schistosomiasis. *Infect Dis Clin North Am* **1993**, 7 (3), 635-53.
 115. Silva, L. C., et al., Treatment of schistosomal myeloradiculopathy with praziquantel and corticosteroids and evaluation by magnetic resonance imaging: a longitudinal study. *Clin Infect Dis* **2004**, 39 (11), 1618-24.
 116. Ferrari, T. C., et al., Clinical characterization of neuroschistosomiasis due to *Schistosoma mansoni* and its treatment. *Acta Trop* **2008**, 108 (2-3), 89-97.
 117. Molyneux, D. H., et al., "Rapid-impact interventions": how a policy of integrated control for Africa's neglected tropical diseases could benefit the poor. *PLoS Med* **2005**, 2 (11), e336.
 118. Hotez, P. J., et al., Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. *PLoS Med* **2006**, 3 (5), e102.
 119. Silva, I. M., et al., Therapeutic failure of praziquantel in the treatment of *Schistosoma haematobium* infection in Brazilians returning from Africa. *Mem Inst Oswaldo Cruz* **2005**, 100 (4), 445-9.
 120. Wang, W., et al., Is there reduced susceptibility to praziquantel in *Schistosoma japonicum*? Evidence from China. *Parasitology* **2010**, 137 (13), 1905-12.
 121. Melman, S. D., et al., Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of *Schistosoma mansoni*. *PLoS Negl Trop Dis* **2009**, 3 (8), e504.
 122. King, C. H., et al., Chemotherapy-based control of schistosomiasis haematobia. II. Metrifonate vs. praziquantel in control of infection-associated morbidity. *Am J Trop Med Hyg* **1990**, 42 (6), 587-95.
 123. Utzinger, J., et al., Effect of combined treatment with praziquantel and artemether on *Schistosoma japonicum* and *Schistosoma mansoni* in experimentally infected animals. *Acta tropica* **2001**, 80 (1), 9-18.

124. Ngoran, E. K.; Utzinger, J., Randomized, double blind placebo controlled trial of oral artemether for the prevention of patent *S. haematobium* infections *Am J Trop Med Hyg* **2003**, 24-32.
125. Bottieau, E., et al., Antimalarial artesunate-mefloquine versus praziquantel in African children with schistosomiasis: an open-label, randomized controlled trial. *Nat Med* **2024**, 30 (1), 130-137.
126. Mnkugwe, R. H., et al., Efficacy and safety of praziquantel and dihydroartemisinin piperaquine combination for treatment and control of intestinal schistosomiasis: A randomized, non-inferiority clinical trial. *PLoS Negl Trop Dis* **2020**, 14 (9), e0008619.
127. Engels, D., et al., The global epidemiological situation of schistosomiasis and new approaches to control and research. *Acta tropica* **2002**, 82 (2), 139-46.
128. Jordan, P., Schistosomiasis--research to control. *Am J Trop Med Hyg* **1977**, 26 (5 Pt 1), 877-86.
129. Liu, R., et al., What is the role of health education in the integrated strategy to control transmission of *Schistosoma japonicum* in China? *Parasitol Res* **2012**, 110 (5), 2081-2.
130. Wang, L. D., et al., A strategy to control transmission of *Schistosoma japonicum* in China. *N Engl J Med* **2009**, 360 (2), 121-8.
131. Cleland, C. R., et al., Mass drug administration with praziquantel reduces the prevalence of *Schistosoma mansoni* and improves liver morbidity in untreated preschool children. *Trans R Soc Trop Med Hyg* **2014**, 108 (9), 575-81.
132. Chaula, S. A.; Tarimo, D. S., Impact of praziquantel mass drug administration campaign on prevalence and intensity of *Schistosoma haematobium* among school children in Bahi district, Tanzania. *Tanzan J Health Res* **2014**, 16 (1), 1-8.
133. Unrau, G. O., Individual household water supplies as a control measure against *Schistosoma mansoni*. A study in rural St Lucia. *Bulletin of the World Health Organization* **1975**, 52 (1), 1-8.
134. Jobin, W. R., et al., Biological control of *Biomphalaria glabrata* in major reservoirs of Puerto Rico. *Am J Trop Med Hyg* **1977**, 26 (5 Pt 1), 1018-24.
135. Wiegand, R. E., et al., Defining elimination as a public health problem for schistosomiasis control programmes: beyond prevalence of heavy-intensity infections. *Lancet Glob Health* **2022**, 10 (9), e1355-e1359.
136. Olveda, R. M., et al., Schistosomiasis japonica in the Philippines: the long-term impact of population-based chemotherapy on infection, transmission, and morbidity. *J Infect Dis* **1996**, 174 (1), 163-72.
137. Ivy, J. A., et al., Historical perspective: Revisiting the St. Lucia Project, a multi-year comparison trial of schistosomiasis control strategies. *PLoS Negl Trop Dis* **2018**, 12 (1), e0006223.
138. Herwaldt, B. L., et al., Persistence of *Schistosoma haematobium* infection despite multiple courses of therapy with praziquantel. *Clin Inf Dis* **1995**, 20 (2), 309-15.
139. Pearce, E. J., Progress towards a vaccine for schistosomiasis. *Acta tropica* **2003**, 86 (2-3), 309-13.
140. Bergquist, R., et al., Blueprint for schistosomiasis vaccine development. *Acta tropica* **2002**, 82 (2), 183-92.
141. Capron, A., et al., Vaccine strategies against schistosomiasis: from concepts to clinical trials. *International archives of allergy and immunology* **2001**, 124 (1-3), 9-15.
142. Kojima, S., et al., A vaccine trial for controlling reservoir livestock against schistosomiasis japonica. *Proceedings of the 9th International Congress of Parasitology Bologna* **1998**, 489-494.
143. Onkanga, I. O., et al., Impact of two rounds of praziquantel mass drug administration on *Schistosoma mansoni* infection prevalence and intensity: a comparison between community wide treatment and school based treatment in western Kenya. *Int J Parasitol* **2016**.
144. Almeida, G. T., et al., Synergy of omeprazole and praziquantel *in vitro* treatment against *Schistosoma mansoni* adult worms. *PLoS Negl Trop Dis* **2015**, 9 (9), e0004086.
145. Buro, C., et al., Imatinib treatment causes substantial transcriptional changes in adult *Schistosoma mansoni in vitro* exhibiting pleiotropic effects. *PLoS Negl Trop Dis* **2014**, 8 (6), e2923.
146. Basra, A., et al., Efficacy of mefloquine intermittent preventive treatment in pregnancy against *Schistosoma haematobium* infection in Gabon: a nested randomized controlled assessor-blinded clinical trial. *Clin Infect Dis* **2013**, 56 (6), e68-75.

147. Hotez, P. J., et al., Developing vaccines to combat hookworm infection and intestinal schistosomiasis. *Nat Rev Microbiol* **2010**, 8 (11), 814-26.
148. Caldwell, N., et al., Perspective on Schistosomiasis Drug Discovery: Highlights from a Schistosomiasis Drug Discovery Workshop at Wellcome Collection, London, September 2022. *ACS Infect Dis* **2023**, 9 (5), 1046-1055.
149. Acosta, L. P., et al., Immune correlate study on human *Schistosoma japonicum* in a well-defined population in Leyte, Philippines: I. Assessment of 'resistance' versus 'susceptibility' to *S. japonicum* infection. *Acta tropica* **2002**, 84 (2), 127-36.
150. Acosta, L. P., et al., Immune correlate study on human *Schistosoma japonicum* in a well-defined population in Leyte, Philippines: II. Cellular immune responses to *S. japonicum* recombinant and native antigens. *Acta tropica* **2002**, 84 (2), 137-49.
151. Riveau, G., et al., Safety and efficacy of the rSh28GST urinary schistosomiasis vaccine: A phase 3 randomized, controlled trial in Senegalese children. *PLoS Negl Trop Dis* **2018**, 12 (12), e0006968.
152. Diemert, D. J., et al., A randomized, controlled Phase 1b trial of the Sm-TSP-2 Vaccine for intestinal schistosomiasis in healthy Brazilian adults living in an endemic area. *PLoS Negl Trop Dis* **2023**, 17 (3), e0011236.
153. Hotez, P. J., et al., Advancing the Development of a Human Schistosomiasis Vaccine. *Trends Parasitol* **2019**, 35 (2), 104-108.
154. Molehin, A. J., et al., Vaccines for Human Schistosomiasis: Recent Progress, New Developments and Future Prospects. *Int J Mol Sci* **2022**, 23 (4).

34. *Clonorchis sinensis* and *Opisthorchis* spp.

Clonorchis sinensis
(Looss 1907)

Pronunciation: \klō-'nōr-kəs\ \sin-en-səs\

Opisthorchis viverrini
(Poirier 1886)

Pronunciation: \äp-əs-'thōr-kəs\ \vi-ver-rē-nē\

Opisthorchis felineus
(Rivolta 1884/Blanchard 1895)

Pronunciation: \äp-əs-'thōr-kəs\ \fel-in-ē-əs\

Introduction

There are three major food-borne liver flukes of clinical significance: *Clonorchis sinensis* (KLO-nor-kis\ \SIN-en-sis\), *Opisthorchis viverrini* (\AH-pis-thor-kis\ \vi-ver-REE-nee), and *O. felineus* (\AH-pis-thor-kis\ \fel-in-ee-us\). ¹ *C. sinensis* is endemic mainly in China and Korea (North and South), but it is found elsewhere in Southeast Asia and is acquired by eating raw or undercooked freshwater fish. ² *O. viverrini* is endemic to northern Thailand, Vietnam, Cambodia, and Laos, where it causes significant disease. Infection with *O. felineus* occurs in Siberia; a focus has also been identified in Italy. ^{1, 3-6} All three species' biology, pathogenesis, and clinical disease are similar. In most of this chapter, *C. sinensis* will be presented as the model organism for food-borne liver flukes. Differences between *O. viverrini* and *O. felineus* will be discussed as they relate to the clinical diseases they cause.

C. sinensis has numerous reservoir hosts, including dogs and cats. More than 25 million people in the Far East are infected with these food-borne liver flukes, and some estimate that up to one-fourth of Chinese immi-

grants to the United States harbor them, as well.^{2, 7} Approximately 10 million people in northern Thailand are infected with *O. viverrine* and 16 million in the former Union of Soviet Socialist Republics with *O. felineus*.^{8, 9} These liver flukes have been identified as potent inducers of carcinogenesis and significant causes of bile duct cancer (cholangiocarcinoma).^{10, 11}

Historical information

In 1875, James McConnell described the adult fluke in a patient who died at a hospital

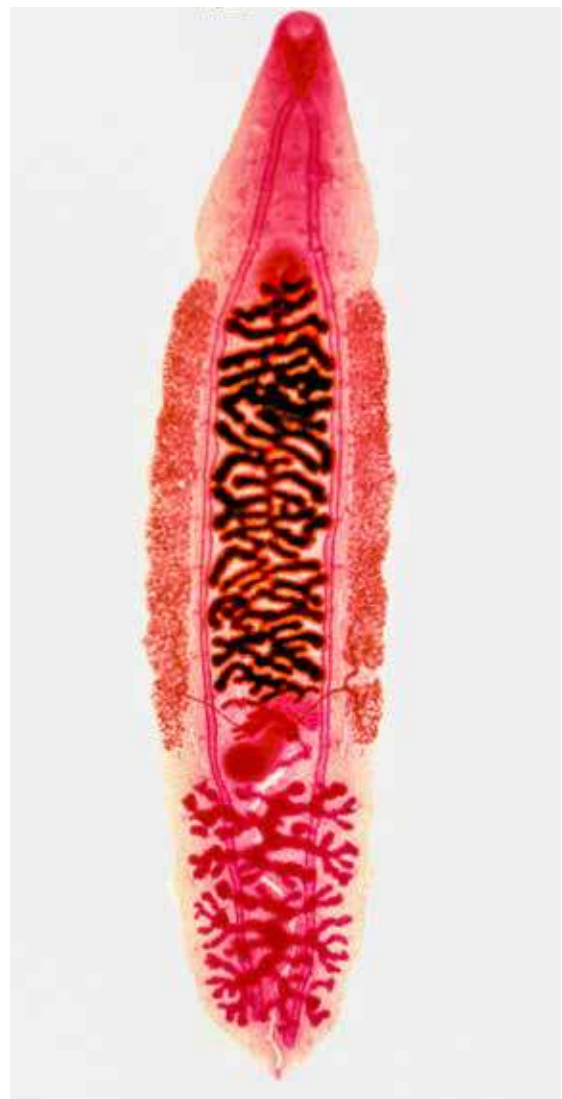
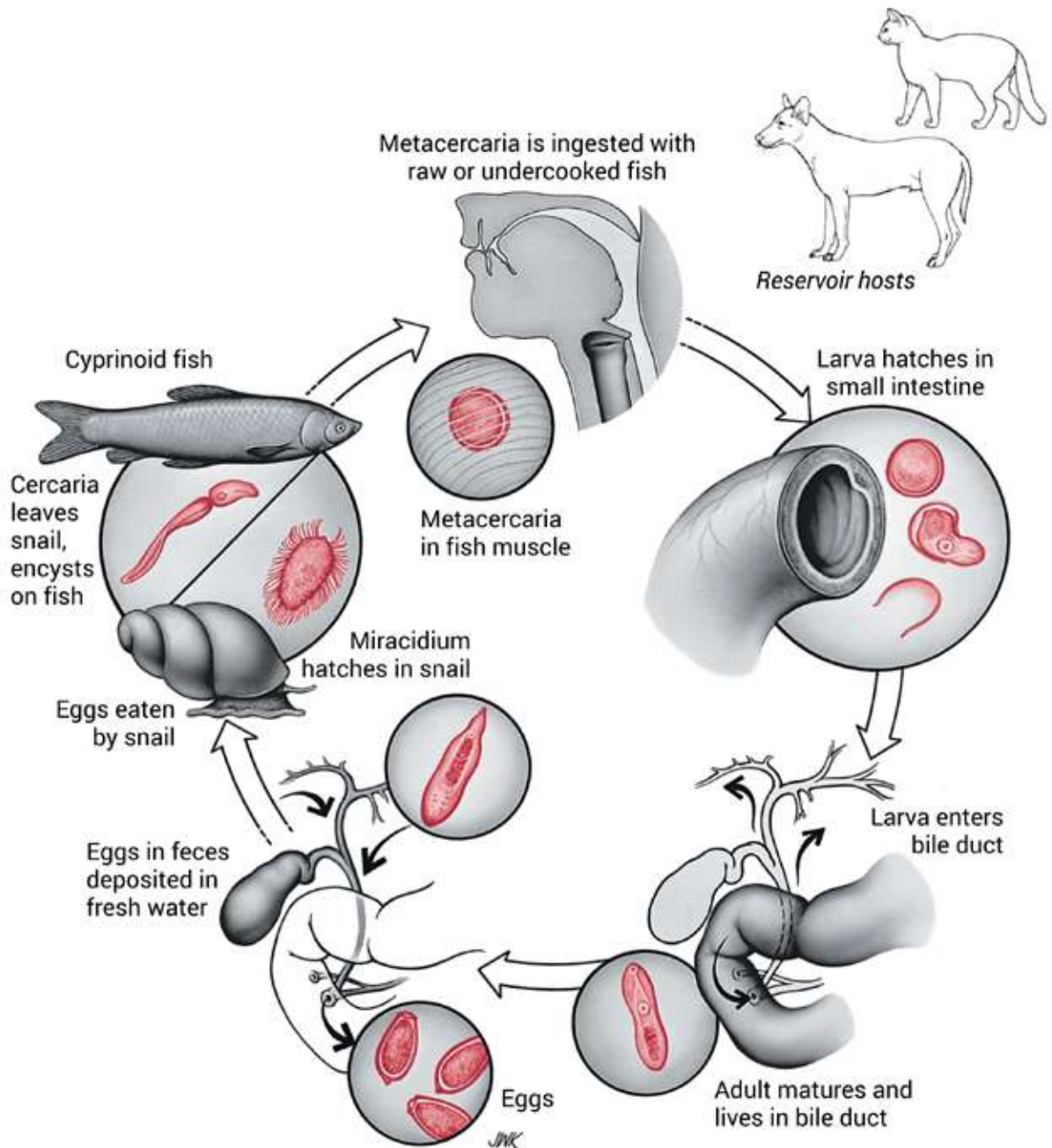
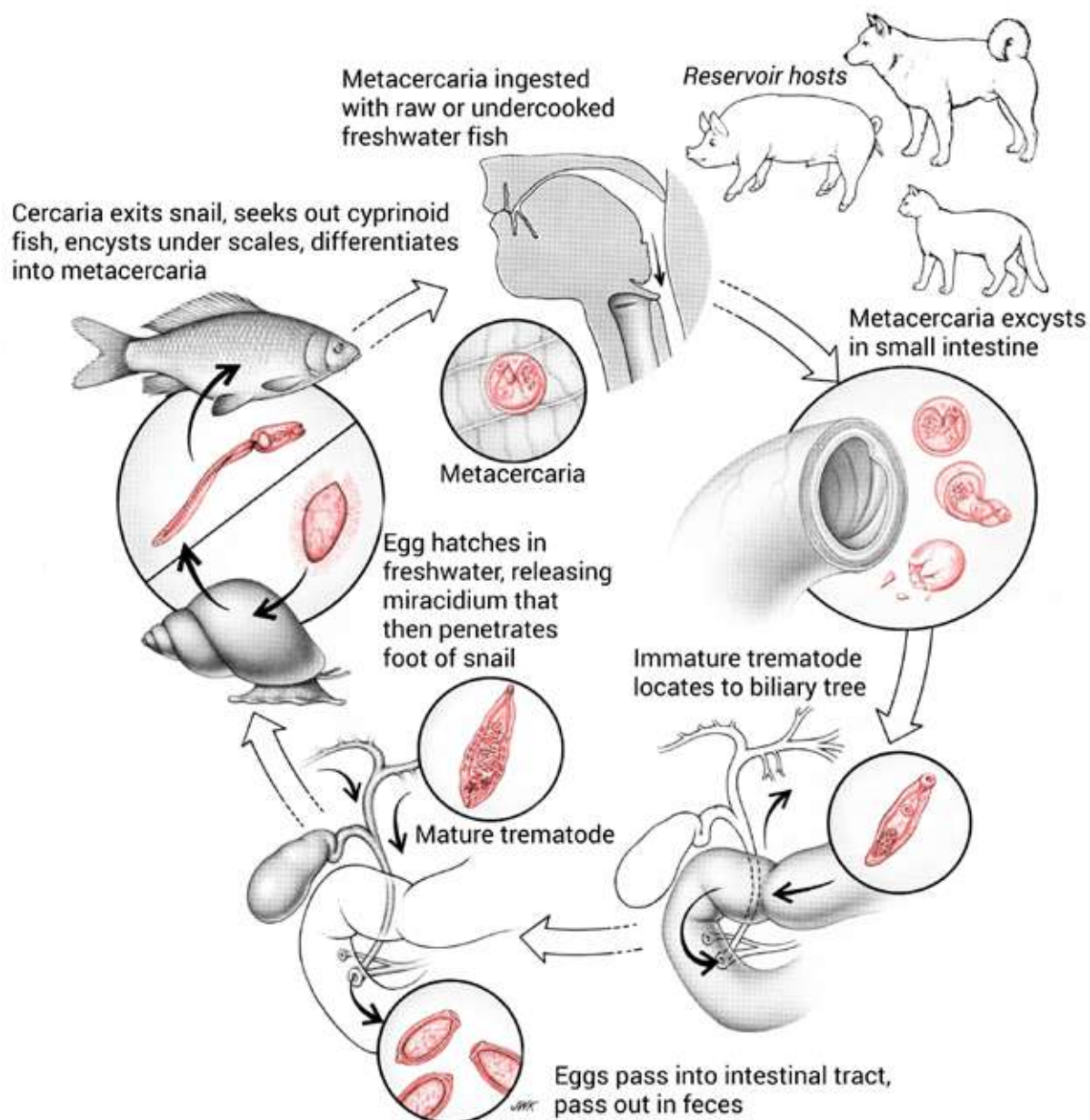


Figure 34.1. An adult *Clonorchis sinensis*. 19 mm x 3.5 mm.

Clonorchis sinensis



Opisthorchis viverrini



in Calcutta, India, and Arthur Looss renamed it *C. sinensis* in 1907.^{7,12} In 1887, Isao Ijima demonstrated that *C. sinensis* infects animals, establishing the concept of reservoir hosts for this parasite.¹³ In 1910, Haraujiro Kobayashi identified freshwater fish as the intermediate vertebrate hosts.¹⁴ In 1918, Masatomo Muto extended these studies in Japan by identifying snails in the genus *Parafossarulus* as the first intermediate host.¹⁵ It is now known that the genus of snails responsible for harboring the intermediate stages of these trematodes varies from region to region, with at least eight different species already described for *C. sinensis*.¹⁶

Life Cycle

Infection begins when the definitive host ingests a raw, pickled, salted, smoked, frozen or undercooked fish or crustacean harboring the metacercaria (Fig. 34.2).^{17,18} Multiple species of freshwater fish may harbor this parasite previously referred to as fish-borne liver flukes; today grouped with food-borne liver flukes because it has been discovered that freshwater crustaceans, such as shrimp, may also act as an intermediate host.¹⁷

The ingested larval stage excysts in the small intestine and transforms into the immature fluke. The flukes then enter the biliary system through the ampulla of Vater and migrate up

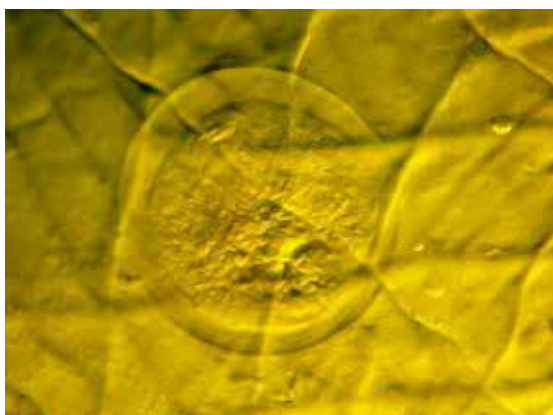


Figure 34.2. Metacercaria of *C. sinensis*, in situ, under the scales of a grass carp. 165 μ m.

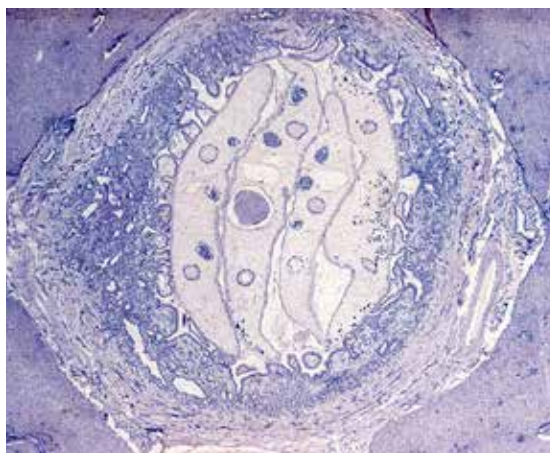


Figure 34.3. Histological section of adults of *C. sinensis* in bile duct.

the bile duct (Fig. 34.3), remaining there and growing to adulthood within several weeks.¹⁹ The method of travel up the bile ducts to reach intrahepatic sites in the biliary system is in contrast to the migration of *Fasciola hepatica*, which penetrates Glisson's capsule and then migrates through the liver parenchyma before ending up in the extrahepatic biliary ducts.¹⁹

The mature parasite (Fig. 34.1) measures 20 mm by 3.5 mm and lives in the lumen of the bile duct, feeding on the epithelium. Each parasite may live for up to 26 years in the biliary system.²⁰ Self-fertilization is the norm since each worm has both male and female reproductive organs. Single worms can produce eggs without the requirement of finding a mate. Egg production follows self-fertiliza-



Figure 34.4. Eggs of *C. sinensis*. 30 μ m x 15 μ m.

tion. Embryonated eggs (Fig. 34.4) pass from the common bile duct into the small intestine and are excreted with the feces. These eggs must reach freshwater to continue the life cycle. In experimental infections, adult worms produce 1000–4000 eggs per day, and in human infection, egg production is about 4000 eggs per day per worm.²¹

Eggs are eaten by the intermediate host snail (in most of Asia, *Parafossarulus* spp.), stimulating the miracidium to hatch. The miracidia then penetrate the intestinal wall. Flukes undergo a process of asexual maturation with development to the sporocyst, then the redia stage, then cercariae. Cercariae emerge from the snail approximately 95 days later.¹⁸ The cercarial stage is highly motile, and when it encounters an appropriate host, such as a cyprinoid fish, it encysts under the scales, transforming into the metacercaria. Encystment can also occur under the exoskeleton of various freshwater crustacea (e.g., crabs, crayfish, and shrimp), completing the life cycle.¹⁷

Cellular and Molecular Pathogenesis

Adult worms induce eosinophilic inflammatory reactions after they attach to the bile duct and begin feeding.^{22,23} In heavy infection, these changes may lead to desquamation of the biliary epithelium, formation of crypts, and metaplasia.^{24,25} *C. sinensis* elicits the production of specific IgE antibodies in serum and bile.²⁶ Chronic clonorchiasis and opisthorchiasis infections elicit reactions resulting in intermittent obstruction of the biliary tree and the introduction of pyogenic bacteria into the infection sites.²⁷ Chronic liver fluke infection can result in recurrent ascending cholangitis and pancreatitis through this process.^{16, 28–31} Over time, these food-borne trematodes in the biliary tree may result in squamous metaplastic changes that lead to cholangiocarci-

noma.³² This is particularly true of heavy *O. viverrini* infections in Thailand, which can be associated with a 15-fold increase in the risk of developing this unusual form of cancer. A much higher percentage of patients who died of cholangiocarcinoma had coexistent opisthorchiasis than did those who died of other causes.²⁵ The molecular basis of helminth-induced carcinogenesis is being unraveled.³³

Clinical Disease

The symptoms seen in acutely and chronically infected patients with these food-borne trematodes are determined largely by the total number of adult worms present. In light infections initiated by a few metacercariae, patients are often asymptomatic. In contrast, patients harboring large numbers of worms may present with right upper quadrant abdominal discomfort and tenderness, nausea, diarrhea, and headache.³⁴

Heavy chronic infections can result in hepatomegaly, tenderness in the right upper quadrant, and eosinophilia. They can also facilitate the sequestration of pyogenic bacteria behind areas of intrahepatic biliary narrowing, causing recurrent ascending cholangitis and pancreatitis.^{16, 28–31}

Very heavy infections can lead to anorexia, cachexia, and weight loss with elevated alkaline phosphatase but normal hepatic transaminase levels. Small differences in the anatomy explain the parasites' anatomical preference for the left lobe of the liver. Cholangiocarcinoma (bile duct carcinoma) is a long-standing sequelae due to chronic fibrosis and infection. It has a high mortality rate in Asia.

Diagnosis (see Clinical Appendix)

Four weeks after initial infection, eggs of these food-borne trematodes start to be

released into human feces. A microscopic examination of a concentrated sample of feces is the definitive gold standard test.⁷ In light infection, eggs may be detectable in concentrated specimens. During periods of biliary obstruction, when patients present for care, eggs may not be detected in the stool.³⁵ Flukes may be detected by endoscopic retrograde cholangiopancreatography (ERCP). Flukes in the biliary tract may also be observed using ultrasound, CT, MRI, and cholangiography.³⁶⁻⁴⁰ Various serological tests, including Western blot and ELISA, are available but cannot distinguish between current and past infections and may cross-react with other parasitic diseases.^{41,42} NAATs (e.g., loop-mediated isothermal amplification (LAMP)) have been developed to improve the sensitivity of egg detection in stool. Still, they are not used routinely in the clinics where most of these cases are seen.⁴³⁻⁴⁶

Treatment (see Clinical Appendix)

Praziquantel is the drug of choice for treating *C. sinensis*, *O. viverrini*, and *O. felineus*.⁴⁷ Albendazole is also effective for *C. sinensis* but shows only modest efficacy for treating *O. viverrini* and *O. felineus*.^{48,49}

Prevention and Control

C. sinensis is one of four food-borne trematodes with recommended plans for elimination outlined in The 2020 WHO NTD Roadmap.⁵⁰ Obstacles to elimination include underdiagnosis, an incomplete understanding of the epidemiology, and ingrained cultural dietary habits.⁵¹ Ingestion of contaminated raw, undercooked, pickled, frozen, salted, smoked, or dried freshwater fish or crustaceans is the source of infection with *C. sinensis* and its close relatives. In many parts of Asia, it is a common practice to grind fish

(inadvertently containing metacercariae) into a paste with spices and condiments to produce a dish roughly equivalent to ceviche. This concoction is a prime source of liver fluke infection. Cooking contaminated fish and crustaceans is the most effective way to prevent infection with this parasite.¹⁶ At least one form of biliary carcinoma is preventable by changes in eating habits. Centuries-old culinary preferences in most endemic areas do not allow for this possibility. In some areas, ingesting raw fish has essential roles in traditional, cultural, and religious contexts.⁷ There is also a long-held myth that hot spices and alcohol consumption, along with the raw fish, will be protective, but there is no evidence to support this belief.⁷

The advent of large-scale aquaculture of grass carp and related fishes in areas where fecal contamination of the ponds from infected hosts occurs regularly results in the establishment of infection in the fish population.¹⁶

³¹ Animal reservoirs make control of this parasite difficult at best. Chemical treatment or proper composting techniques that create a thermophilic phase are recommended to process human feces destined for fertilizer.⁵²

Molluscicides alone have not been used successfully to eradicate intermediate snail hosts, and there are concerns about the impact of their use on the environment.⁵³ The combination of regular draining of ponds and molluscicides has been moderately effective in controlling infection in fish. The 2020 WHO NTD Roadmap recommends an integrated program for treating infected humans, keeping animal reservoirs such as dogs away from fish ponds, and thoroughly cooking food. Human vaccines have been studied, and a vaccination strategy targeting freshwater fish has been tested. However, the absence of a vaccine limits current control and elimination efforts.

References

1. Petney, T. N., et al., The zoonotic, fish-borne liver flukes *Clonorchis sinensis*, *Opisthorchis felinus* and *Opisthorchis viverrini*. *Int J Parasitol* **2013**, *43* (12-13), 1031-46.
2. Stauffer, W. M., et al., Biliary liver flukes (Opisthorchiasis and Clonorchiasis) in immigrants in the United States: often subtle and diagnosed years after arrival. *Journal of travel medicine* **2004**, *11* (3), 157-9.
3. Sripa, B., et al., Food-borne trematodiasis in Southeast Asia epidemiology, pathology, clinical manifestation and control. *Adv Parasitol* **2010**, *72*, 305-50.
4. Kruthong, S., et al., *Opisthorchis viverrini* infection in rural communities in northeast Thailand. *Trans Med Hyg* **1987**, *81*, 1-414.
5. Giboda, M., et al., Human *Opisthorchis* and *Haplorchis* infections in Laos. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1991**, *85* (4), 538-40.
6. Wunderink, H. F., et al., Foodborne trematodiasis and *Opisthorchis felinus* acquired in Italy. *Emerg Infect Dis* **2014**, *20* (1), 154-5.
7. Qian, M. B., et al., Clonorchiasis. *Lancet* **2015**.
8. Schwartz, D. A., Cholangiocarcinoma associated with liver fluke infection: a preventable source of morbidity in Asian immigrants. *The American journal of gastroenterology* **1986**, *81* (1), 76-9.
9. WHO, Control of food-borne trematode infections: Report of a WHO study group. *Report Series No 849 Organization* **1995**.
10. Sithithaworn, P., et al., Roles of liver fluke infection as risk factor for cholangiocarcinoma. *J Hepatobiliary Pancreat Sci* **2014**, *21* (5), 301-8.
11. Sripa, B., et al., The tumorigenic liver fluke *Opisthorchis viverrini*--multiple pathways to cancer. *Trends Parasitol* **2012**, *28* (10), 395-407.
12. McConnell, J. F. P., Anatomy and pathological relations of a new species of liver fluke. *Lancet* **1875**, *2* 271-274.
13. Ijima, I.; J., Notes on *Diastoma endemicum*. *Baelz Sci Imperial Univ Jpn* **1887**, *1*, 47-50.
14. Kobayashi, H., On the life cycle and morphology of *Clonorchis sinensis*. *Centr BakterParasit InfectKrank* **1914**, *75*, 299-317.
15. Muto, S., On the primary intermediate host of *Clonorchis sinensis*. *Chuo Igakakai Zasshi* **1918**, *25*, 49-52.
16. Lun, Z.-R., et al., Clonorchiasis: a key foodborne zoonosis in China. *The Lancet. Infectious diseases* **2005**, *5* (1), 31-41.
17. Yang, L. D., et al., [An epidemiological study on *clonorchiasis sinensis* in Hubei Province]. *Zhonghua Yu Fang Yi Xue Za Zhi* **1994**, *28* (4), 225-7.
18. Liang, C., et al., [Experimental establishment of life cycle of *Clonorchis sinensis*]. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* **2009**, *27* (2), 148-50.
19. Kim, T. I., et al., Tracing of the Bile-chemotactic migration of juvenile *Clonorchis sinensis* in rabbits by PET-CT. *PLoS Negl Trop Dis* **2011**, *5* (12), e1414.
20. Attwood, H. D.; Chou, S. T., The longevity of *Clonorchis sinensis*. *Pathology* **1978**, *10* (2), 153-6.
21. Kim, J. H., et al., Correlation between discharged worms and fecal egg counts in human clonorchiasis. *PLoS Negl Trop Dis* **2011**, *5* (10), e1339.
22. Sun, T., Pathology and immunology of *Clonorchis sinensis* infection of the liver. *Annals of clinical and laboratory science* **1984**, *14* (3), 208-15.
23. Yen, C. M., et al., Antibodies of different immunoglobulin isotypes in serum and bile of patients with clonorchiasis. *Annals of tropical medicine and parasitology* **1992**, *86* (3), 263-9.
24. Pungpak, S., et al., Tumour markers in the detection of opisthorchiasis-associated cholangiocarcinoma. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1991**, *85* (2), 277-9.
25. Srivatanakul, P., et al., Epidemiology of liver cancer: an overview. *Asian Pacific journal of cancer prevention : APJCP* **2004**, *5* (2), 118-25.
26. Yong, T. S., et al., Identification of IgE-reacting *Clonorchis sinensis* antigens. *Yonsei medical journal* **1999**, *40* (2), 178-83.

27. Ho, C. S.; Wesson, D. E., Recurrent pyogenic cholangitis in Chinese immigrants. *The American journal of roentgenology, radium therapy, and nuclear medicine* **1974**, 122 (2), 368-74.
28. McFadzen, A. J. S., et al., Acute pancreatitis due to *Clonorchis sinensis*. *Trans Trop Med Hyg* **1966**, 60.
29. Haswell-Elkins, M. R., et al., Cross-sectional study of *Opisthorchis viverrini* infection and cholangiocarcinoma in communities within a high-risk area in northeast Thailand. *International journal of cancer* **1994**, 59 (4), 505-9.
30. Thamavit, W., et al., Effects of dimethylnitrosamine on induction of cholangiocarcinoma in *Opisthorchis viverrini*-infected Syrian golden hamsters. *Cancer research* **1978**, 38 (12), 4634-9.
31. Wang, K. X., et al., Clinical and epidemiological features of patients with clonorchiasis. *World J Gastroenterol* **2004**, 10 (3), 446-8.
32. Okuda, K., et al., Clinical aspects of intrahepatic bile duct carcinoma including hilar carcinoma: a study of 57 autopsy-proven cases. *Cancer* **1977**, 39 (1), 232-46.
33. Brindley, P. J., et al., Why does infection with some helminths cause cancer? *Trends Cancer* **2015**, 1 (3), 174-182.
34. Liang, S. D., et al., [Analysis on 2175 admitted cases of *clonorchiasis sinensis* in Guigang City]. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* **2008**, 26 (5), 374-5.
35. Joo, K. R.; Bang, S. J., A bile based study of *Clonorchis sinensis* infections in patients with biliary tract diseases in Ulsan, Korea. *Yonsei Med J* **2005**, 46 (6), 794-8.
36. Choi, D.; Hong, S. T., Imaging diagnosis of clonorchiasis. *Korean J Parasitol* **2007**, 45 (2), 77-85.
37. Jeong, Y. Y., et al., MR imaging findings of clonorchiasis. *Korean J Radiol* **2004**, 5 (1), 25-30.
38. Lim, J. H., Radiologic findings of clonorchiasis. *AJR Am J Roentgenol* **1990**, 155 (5), 1001-8.
39. Choi, B. I., et al., CT findings of clonorchiasis. *AJR Am J Roentgenol* **1989**, 152 (2), 281-4.
40. Lim, J. H., et al., Clonorchiasis: sonographic findings in 59 proved cases. *AJR Am J Roentgenol* **1989**, 152 (4), 761-4.
41. Kim, S. I., A *Clonorchis sinensis*-specific antigen that detects active human clonorchiasis. *The Korean journal of parasitology* **1998**, 36 (1), 37-45.
42. Kim, C. S., et al., Immunodiagnosis of clonorchiasis using a recombinant antigen. *Korean J Parasitol.* **1998**, 36, 183-90.
43. Kim, E. M., et al., Detection of *Clonorchis sinensis* in stool samples using real-time PCR. *Ann Trop Med Parasitol* **2009**, 103 (6), 513-8.
44. Parvathi, A., et al., Development and evaluation of a polymerase chain reaction (PCR) assay for the detection of *Opisthorchis viverrini* in fish. *Acta Trop* **2008**, 107 (1), 13-6.
45. Umesha, K. R., et al., *Opisthorchis viverrini*: detection by polymerase chain reaction (PCR) in human stool samples. *Exp Parasitol* **2008**, 120 (4), 353-6.
46. Arimatsu, Y., et al., Rapid detection of *Opisthorchis viverrini* copro-DNA using loop-mediated isothermal amplification (LAMP). *Parasitol Int* **2012**, 61 (1), 178-82.
47. Hsu, C. C.; Kron, M. A., Clonorchiasis and praziquantel. *Archives of internal medicine* **1985**, 145 (6), 1002-3.
48. Liu, Y. H., et al., Experimental and clinical trial of albendazole in the treatment of *Clonorchiasis sinensis*. *Chin Med J (Engl)* **1991**, 104 (1), 27-31.
49. Pungpark, S., et al., Albendazole in the treatment of opisthorchiasis and concomitant intestinal helminthic infections. *Southeast Asian J Trop Med Public Health* **1984**, 15 (1), 44-50.
50. Organization, W. H., Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva. Licence: CC BY-NC-SA 3.0 IGO **2020**.
51. Tidman, R., et al., Global prevalence of 4 neglected foodborne trematodes targeted for control by WHO: A scoping review to highlight the gaps. *PLoS Negl Trop Dis* **2023**, 17 (3), e0011073.
52. Kone, D., et al., Helminth eggs inactivation efficiency by faecal sludge dewatering and co-composting in tropical climates. *Water Res* **2007**, 41 (19), 4397-402.
53. Sithithaworn, P., et al., The current status of opisthorchiasis and clonorchiasis in the Mekong Basin. *Parasitol Int* **2012**, 61 (1), 10-6.
54. Wang, X., et al., Surface display of *Clonorchis sinensis* enolase on *Bacillus subtilis* spores potentializes an oral vaccine candidate. *Vaccine* **2014**, 32 (12), 1338-45.

35. *Fasciola hepatica*

(Linnaeus 1758)

Pronunciation: \fə-'sē-ə-lə\hi-'pa-ti-kə\

Introduction

Fasciola hepatica (\fah-SEE-ow-lah\he-pa-tee-KUH), the sheep liver fluke, is acquired by eating contaminated leafy wild plants (e.g., watercress) that grow at the littoral zone of standing bodies of freshwater. Fascioliasis is a zoonosis that infects wild animals and livestock of all kinds and is endemic worldwide.^{1,2} A few cases have been reported in the United States and Australia.^{3,4} New Zealand has eliminated *F. hepatica* by employing aggressive control programs began in the 1960s and '70s.⁵ *F. hepatica* infects millions worldwide and has been reported in over 50 countries.⁶⁻⁹



Figure 35.1. Adult of *Fasciola hepatica*. 30mm x 14 mm.

The intensity of infection in humans is always associated with animal husbandry.¹⁰ There is a high prevalence in areas of South America, such as Peru and Bolivia, particularly in the northwestern altiplano of Bolivia near Lake Titicaca. *Fasciola gigantica* is a closely related species that infects cattle and wild herbivores in Africa and Asia and can, on rare occasions, also infect humans.¹²⁻¹⁵

Historical Information

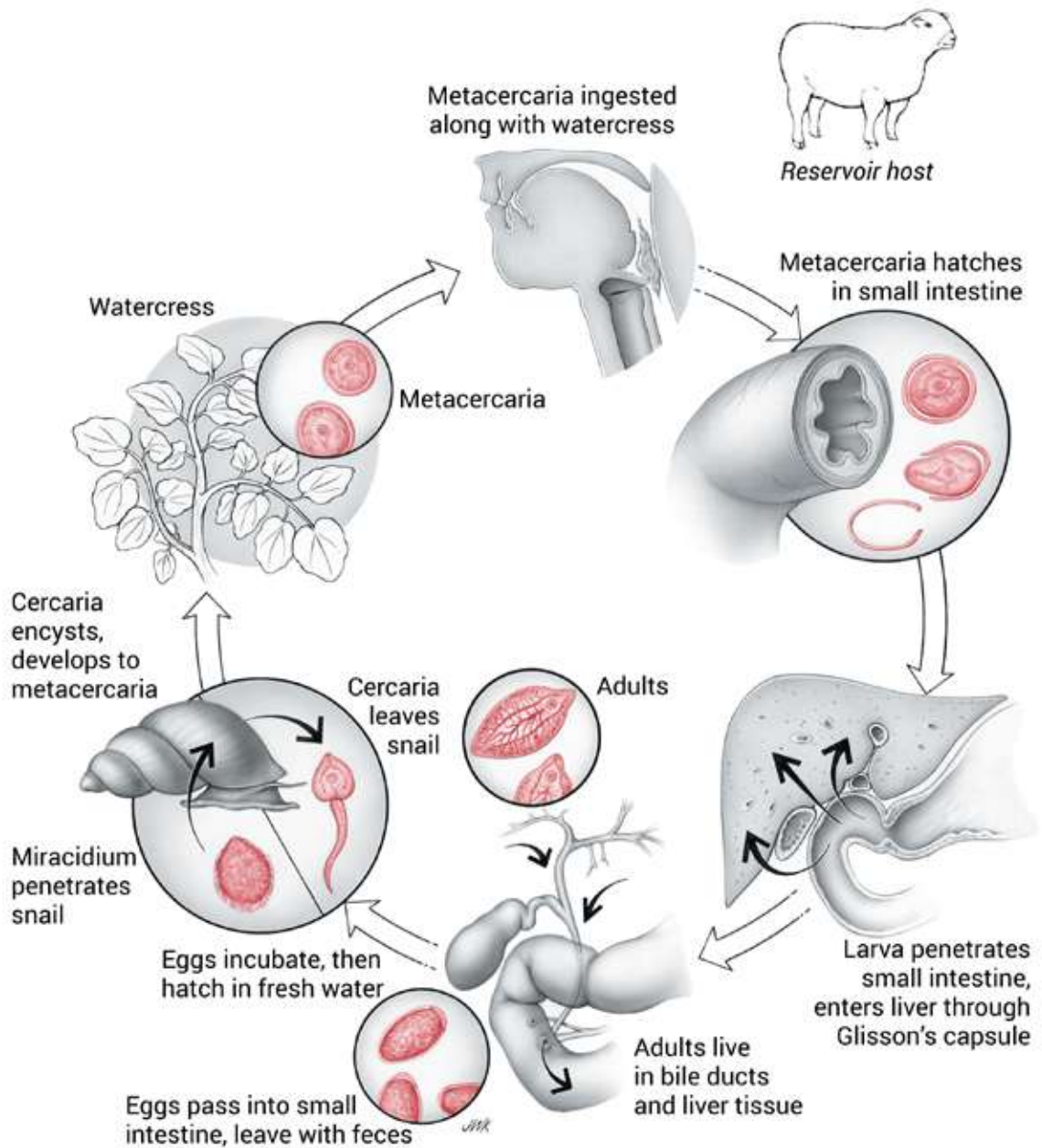
The writings of Jean de Brie in 1379 indicate that shepherds not only knew of the infection but also strongly suspected that contaminated watercress was a source of the parasite.¹⁶ In 1684, Francesco Redi described the adult parasite he obtained from a rabbit.¹⁷ In 1758, Carl Linnaeus named this parasite *Fasciola hepatica*.^{18,19} In 1881, Friedrich Leuckart and Algernon Thomas independently described most of the biological aspects of its life cycle.^{20,21} In 1892, Adolfo Lutz conducted experiments on guinea pigs, proving that the adult parasite was acquired by swallowing the infective stage.²²

Life Cycle

Infection is initiated by ingesting encysted metacercariae firmly attached to littoral vegetation, particularly watercress, in standing bodies of freshwater (e.g., farm ponds).²³ When swallowed, they excyst in the small intestine, penetrate the intestinal wall, and migrate in the peritoneal cavity to the surface of the liver. Metacercariae penetrate Glisson's capsule and enter the parenchymal tissue of the liver. Metacercaria migrate through the liver, causing necrosis and fibrosis. Only a small number of the infective stage reach the biliary tree to develop to sexual maturity.

Adults take up to 4 months to mature. The adult worm is large, measuring 35 mm by 15 mm (Fig. 35.1), and can live in the biliary tree for over a decade.

Fasciola hepatica



Immature worms feed on liver parenchymal tissue (*foie gras d'homme*), and adults feed on epithelial cells lining the bile ducts. The juveniles burrow through the liver, aided by their muscular oral suckers, creating tunnels into which are deposited eggs and waste products. Self-fertilization leads to egg production.

Fertilized, unembryonated eggs (Fig. 35.3) pass out of the liver through the common duct, enter the small intestine, and become included in the fecal mass. Eggs must be deposited in freshwater to embryonate, which may take 9–15 days. The miracidium is stimulated to hatch by exposure to direct sunlight. It is a free-swimming organism after emerging from the egg until it finds its appropriate snail host. The most common snail species for *F. hepatica* is *Lymnea truncatula*. How-

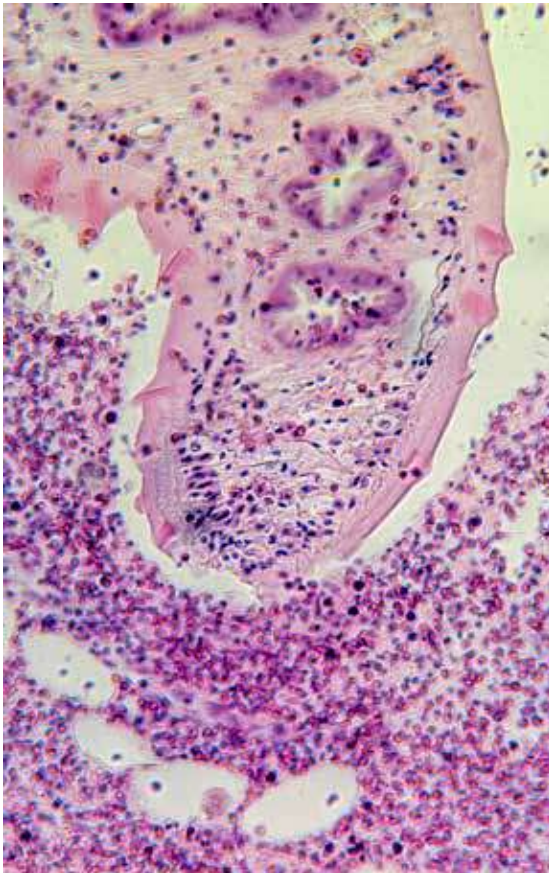


Figure 35.2. Histological section of adult *F. hepatica* in liver.



Figure 35.3. Egg of *F. hepatica*. 140 μm x 85 μm .

ever, many other species of Lymneid snails (e.g., *Fossaria modicella*) support the growth and development of this fluke worldwide. The miracidium penetrates the snail's body wall and reaches the hepatopancreas. It then undergoes sequential development, first into sporocysts, then rediae, and then cercariae (Fig. 35.4), which emerge from the snail. These attach to the surfaces of littoral vegetation, where they encyst. Within the cyst, they transform into the environmentally resistant, infective stage, the metacercaria. This stage can remain infective for several months.

Ingested metacercariae sometimes reach tissues other than the liver (e.g., brain, kidney).²⁴

²⁵ In this case, they become aberrant infections and pass eggs that cannot leave the body.

Cellular and Molecular Pathogenesis

Adult *F. hepatica* secrete large quantities of proline, which stimulates bile epithelial cells to divide and hypertrophy, creating the “lawn” of cells on which the fluke periodically grazes, presumably with the aid of its muscular oral sucker and secreted proteases.²⁶

²⁷ While moving through liver tissue, this fluke creates trauma. Tunnels and abscesses form that fill with necrotic cell debris and worm excreta.



Figure 35.4. Cercaria of *F. hepatica*. 100 μ m.

Fascioliasis induces high levels of circulating eosinophils throughout the infection period.²⁸

29

Halzoun (pharyngitis and laryngeal edema) is a condition specific to the Middle East associated with the consumption of raw sheep liver. Although previously attributed to *F. hepatica*, it may, in most cases, be due to other parasitic infections.^{30,31}

Clinical Disease

Individuals may develop symptoms related to the migration of the immature worms within a month of becoming infected.²⁹ Many infected persons are asymptomatic during this early phase. At the same time, symptomatic patients may report fever, pain in the right upper quadrant of the abdomen, headache, generalized malaise, myalgia, weight

loss, and urticaria.³² Circulating eosinophilia is a prominent feature. Prominent radiographic findings on contrast CT have been reported as “hypoattenuating tracts” that follow the path of helminth invasion from the liver capsule.³³ Symptoms usually develop 6–12 weeks after exposure and generally last for about six weeks. In heavy infections, the liver can be enlarged and tender. A right-sided pleural effusion with eosinophilia has been described.^{34,35}

Acute disease tends to be proportional to the number of ingested metacercariae. Chronic disease is usually proportional to the number of adult worms in the biliary system. During the chronic stage of the disease, dull pain and obstruction of bile ducts can occur. There are usually no changes in liver function tests, and jaundice is not a usual finding but has been reported.³² The gallbladder may become severely damaged in heavy infection. Fasciola in sites other than the liver may cause no symptoms or be present as a small tumor mass. If the parasite invades the brain, it can induce focal neurological abnormalities.

Diagnosis (see Clinical Appendix)

Diagnosis begins with a clinical suspicion of exposure to *F. hepatica* from a carefully obtained history. Mature adults are present in the biliary tree after four months of maturation in the liver and release eggs into the stool. Serology usually becomes positive during acute infection while the juvenile worms migrate through the liver parenchyma. Most patients will present at this stage with high levels of circulating eosinophils.²⁹ Serological tests can help determine the diagnosis at this stage of the infection and have excellent sensitivity and specificity.^{36–38} *F. hepatica* circulating antigen tests with excellent sensitivity and specificity are available and correlate with the burden of infection.^{39,40}

When mature flukes are present in the biliary system, microscopic identification of eggs in the stool is a definitive method of diagnosis. Eggs may be detected in the feces, bile aspirates, or duodenal aspirates. These unembryonated eggs (Fig. 35.3) are yellow-brown and measure 130–150 μm by 60–90 μm wide.

Imaging techniques such as ultrasonography, CT, cholangiography, endoscopic cholangiopancreatography (ERCP), and MRI may be helpful in diagnosing fascioliasis.^{41–44} During acute disease, linear tracts can be seen in the liver, while filling defects and adult flukes can be visualized in the biliary system during chronic disease.

Treatment (see Clinical Appendix)

Triclabendazole is the drug of choice for treating infection with *Fasciola hepatica*.²⁹
^{45–48} Although triclabendazole is not FDA-approved or generally available in the United States, it can be obtained from the Centers for Disease Control (CDC) under an investigational protocol. Resistance to triclabendazole has emerged and was reported in a German study of sheep farms.⁴⁹ Praziquantel, which is excellent for treating infections with other flukes, is not effective against *Fasciola*, nor are mebendazole, albendazole, or artesunate.^{50–52} Nitazoxanide appears to be an inferior but alternate therapy with some demonstrated efficacy.^{53, 54} Successfully treated patients will develop negative serologies 6–12 months

after clearing their parasites.

Prevention and Control

F. hepatica is a food-borne trematode included in the 2020 WHO NTD Roadmap for elimination. Awareness of this disease is low in endemic populations, and global advocacy coordinating its elimination does not exist. Control may be achieved through periodic draining of ponds, which reduces littoral plant growth to a minimum. Protecting freshwater supplies and regularly surveying herds and herders for the presence of the parasites can achieve further control of the spread of fascioliasis in domestic animals. In this regard, an ELISA test detected experimentally and naturally-infected calves with a high degree of sensitivity and specificity.³⁷ Appropriate treatment in both groups is warranted when infections are detected. Snail elimination with molluscicides has not been successful. Education of farm personnel regarding the mode of acquisition of the infection is essential to eliminating transmission due to human fecal contamination of freshwater aquatic habitats.

Vaccines have been developed for veterinary use that have demonstrated some degree of efficacy in reducing egg production and worm burdens. Despite significant advances in vaccine development, no human or animal vaccine is currently in use.⁵⁶

References

1. Mas-Coma, M. S., et al., Epidemiology of human fascioliasis: a review and proposed new classification. *Bull World Health Organ* **1999**, 77 (4), 340–6.
2. Bosnak, V. K., et al., Evaluation of patients diagnosed with fascioliasis: A six-year experience at a university hospital in Turkey. *J Infect Dev Ctries* **2016**, 10 (4), 389–94.
3. Weisenberg, S. A.; Perlada, D. E., Domestically acquired fascioliasis in northern California. *Am J Trop Med Hyg* **2013**, 89 (3), 588–91.
4. Sivagnanam, S., et al., Hepatic lesions and eosinophilia in an urban dweller. *Liver Int* **2014**, 34 (4), 643.

5. Charleston, W. A.; McKenna, P. B., Nematodes and liver fluke in New Zealand. *N Z Vet J* **2002**, *50* (3 Suppl), 41-7.
6. Lukumbagire, A. H., et al., Diagnosis of human fascioliasis in Arusha region, northern Tanzania by microscopy and clinical manifestations in patients. *BMC Infect Dis* **2015**, *15* (1), 578.
7. Nyindo, M.; Lukumbagire, A. H., Fascioliasis: An Ongoing Zoonotic Trematode Infection. *Biomed Res Int* **2015**, *2015*, 786195.
8. Keiser, J.; Utzinger, J., Food-borne trematodiasis. *Clin Microbiol Rev* **2009**, *22* (3), 466-83.
9. Mas-Coma, S., Epidemiology of fascioliasis in human endemic areas. *J Helminthol* **2005**, *79* (3), 207-16.
10. Esteban, J.-G., et al., Hyperendemic fascioliasis associated with schistosomiasis in villages in the Nile Delta of Egypt. *The American journal of tropical medicine and hygiene* **2003**, *69* (4), 429-37.
11. Hillyer, G. V., et al., Use of the Falcon assay screening test--enzyme-linked immunosorbent assay (FAST-ELISA) and the enzyme-linked immunoelectrotransfer blot (EITB) to determine the prevalence of human fascioliasis in the Bolivian Altiplano. *The American journal of tropical medicine and hygiene* **1992**, *46* (5), 603-9.
12. Menon, P., et al., Biliary *Fasciola gigantica* infestation in a nonendemic area - An intraoperative surprise. *J Pediatr Surg* **2015**, *50* (11), 1983-6.
13. Fang, W., et al., [Case report: *Fasciola gigantica* infection treated by triclabendazole]. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* **2013**, *31* (2), Inside front page.
14. Bestas, R., et al., Cholestasis caused by *Fasciola gigantica*. *Turkiye Parazitolo Derg* **2014**, *38* (3), 201-4.
15. Chen, J. X., et al., An outbreak of human *Fascioliasis gigantica* in Southwest China. *PloS one* **2013**, *8* (8), e71520.
16. de Brie, J., Le Bon Berger ou le Vray Regime et Gouvenement de Bergers et Bergeres: Compose par le Rustique Jehan de Brie le Bon Berger (1379). Isidor Liseux, Paris. **1879**.
17. Redi, F., Osservazioni di Francesco Redi. *Intorno Agli Animali Viventi che si Trovano Negli Animali Viventi Piero Matini Florence* **1684**.
18. Lima Wdos, S., et al., Occurrence of *Fasciola hepatica* (Linnaeus, 1758) infection in Brazilian cattle of Minas Gerais, Brazil. *Rev Bras Parasitol Vet* **2009**, *18* (2), 27-30.
19. Cox, F. E., History of human parasitology. *Clin Microbiol Rev* **2002**, *15* (4), 595-612.
20. Leuckart, F. R., Zur Entwicklungsgeschichte des Lerberegels. *Zool Anz* **1881**, *4*, 641-646.
21. Thomas, A. P. W.; R., J., Report of experiments on the development of the liver fluke, *Fasciola hepatica*. *Soc Engi* **1881**, *17*, 1-28.
22. Lutz, A., Zur Lebensgeschichte des *Distoma hepaticum*. *Zentralbi Bakteriol Parasit* **1892**, *11*, 783-796.
23. Mas-Coma, S., et al., Fascioliasis and other plant-borne trematode zoonoses. *Int J Parasitol* **2005**, *35* (11-12), 1255-78.
24. Catchpole, B. N.; Snow, D., Human ectopic fascioliasis. *Lancet* **1952**, *2* 711-712.
25. Arjona, R., et al., Fascioliasis in developed countries: a review of classic and aberrant forms of the disease. *Medicine* **1995**, *74* (1), 13-23.
26. Modavi, S.; Isseroff, H., *Fasciola hepatica*: collagen deposition and other histopathology in the rat host's bile duct caused by the parasite and by proline infusion. *Experimental parasitology* **1984**, *58* (3), 239-44.
27. Wijffels, G. L., et al., The secreted cathepsin L-like proteinases of the trematode, *Fasciola hepatica*, contain 3-hydroxyproline residues. *The Biochemical journal* **1994**, *299* (Pt 3), 781-90.
28. Demirci, M., et al., Diagnostic importance of serological methods and eosinophilia in tissue parasites. *Journal of health, population, and nutrition* **2002**, *20* (4), 352-5.
29. Saba, R., et al., Human fascioliasis. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **2004**, *10* (5), 385-7.
30. Khalil, G., et al., Halzoun, an allergic pharyngitis syndrome in Lebanon: the trematode *Dicrocoelium dendriticum* as an additional cause. *Acta Trop* **2013**, *125* (1), 115-8.
31. Saleha, A. A., Liver fluke disease (fascioliasis): epidemiology, economic impact and public health significance. *The Southeast Asian journal of tropical medicine and public health* **1991**, *22* Suppl,

- 361-4.
32. Arjona, R., et al., Fascioliasis in developed countries: a review of classic and aberrant forms of the disease. *Medicine (Baltimore)* **1995**, *74* (1), 13-23.
33. Patel, N. U., et al., CT findings of human *Fasciola hepatica* infection: case reports and review of the literature. *Clin Imaging* **2016**, *40* (2), 251-5.
34. Corredoira, J. C., et al., [Eosinophilic pleural effusion caused by *Fasciola hepatica*]. *Enferm Infecc Microbiol Clin* **1990**, *8* (4), 258-9.
35. Moretti, G., et al., [Pleural effusion revealing and a recapitulating *Fasciola hepatica* distomatosis]. *Bord Med* **1971**, *4* (4), 1181-2 passim.
36. O'Neill, J., On the presence of a filaria in "crawcraw." *Lancet* **1875**, *1*, 265-266.
37. Rokni, M. B., et al., Diagnosis of human fasciolosis in the Gilan province of Northern Iran: application of cathepsin L-ELISA. *Diagnostic microbiology and infectious disease* **2002**, *44* (2), 175-9.
38. Gonzales Santana, B., et al., The diagnosis of human fascioliasis by enzyme-linked immunosorbent assay (ELISA) using recombinant cathepsin L protease. *PLoS Negl Trop Dis* **2013**, *7* (9), e2414.
39. Hassan, M. M., et al., Evaluation of circulating *Fasciola* antigens in specific diagnosis of fascioliasis. *J Egypt Soc Parasitol* **2001**, *31* (1), 271-9.
40. Almazan, C., et al., Effect of parasite burden on the detection of *Fasciola hepatica* antigens in sera and feces of experimentally infected sheep. *Vet Parasitol* **2001**, *97* (2), 101-12.
41. Zali, M. R., et al., Liver, spleen, pancreas and kidney involvement by human fascioliasis: imaging findings. *BMC gastroenterology* **2004**, *4*, 15.
42. Cevikol, C., et al., Human fascioliasis: MR imaging findings of hepatic lesions. *Eur Radiol* **2003**, *13* (1), 141-8.
43. Van Beers, B., et al., Hepatobiliary fascioliasis: noninvasive imaging findings. *Radiology* **1990**, *174* (3 Pt 1), 809-10.
44. Dias, L. M., et al., Biliary fascioliasis: diagnosis, treatment and follow-up by ERCP. *Gastrointest Endosc* **1996**, *43* (6), 616-20.
45. el-Karakasy, H., et al., Human fascioliasis in Egyptian children: successful treatment with triclabendazole. *Journal of tropical pediatrics* **1999**, *45* (3), 135-8.
46. Marcos, L. A., et al., Update on hepatobiliary flukes: fascioliasis, opisthorchiasis and clonorchiasis. *Curr Opin Infect Dis* **2008**, *21* (5), 523-30.
47. Marcos, L. A., et al., Natural history, clinicoradiologic correlates, and response to triclabendazole in acute massive fascioliasis. *Am J Trop Med Hyg* **2008**, *78* (2), 222-7.
48. Villegas, F., et al., Administration of triclabendazole is safe and effective in controlling fascioliasis in an endemic community of the Bolivian Altiplano. *PLoS Negl Trop Dis* **2012**, *6* (8), e1720.
49. Kahl, A., et al., Efficacy of flukicides against *Fasciola hepatica* and first report of triclabendazole resistance on German sheep farms. *Int J Parasitol Drugs Drug Resist* **2023**, *23*, 94-105.
50. Fand, Z., et al., Praziquantel and *Fasciola hepatica* infection. *Trans Trop Med Hyg* **1989**, *813*, 83.
51. Hien, T. T., et al., A randomized controlled pilot study of artesunate versus triclabendazole for human fascioliasis in central Vietnam. *Am J Trop Med Hyg* **2008**, *78* (3), 388-92.
52. Cabada, M. M.; White, A. C., Jr., New developments in epidemiology, diagnosis, and treatment of fascioliasis. *Curr Opin Infect Dis* **2012**, *25* (5), 518-22.
53. Rossignol, J. F., et al., Successful treatment of human fascioliasis with nitazoxanide. *Trans R Soc Trop Med Hyg* **1998**, *92* (1), 103-4.
54. Favenne, L., et al., Double-blind, randomized, placebo-controlled study of nitazoxanide in the treatment of fascioliasis in adults and children from northern Peru. *Aliment Pharmacol Ther* **2003**, *17* (2), 265-70.
55. Espino, A. M.; Hillyer, G. V., A novel *Fasciola hepatica* saposinlike recombinant protein with immunoprophylactic potential. *The Journal of parasitology* **2004**, *90* (4), 876-9.
56. Molina-Hernandez, V., et al., *Fasciola hepatica* vaccine: we may not be there yet but we're on the right road. *Vet Parasitol* **2015**, *208* (1-2), 101-11.



Francesco Redi, M.D., Ph.D. (1626–1697)

Redi was a global thinker, an original “myth buster”, and one of the original Renaissance Men of science. He conducted investigations into the origins of life and established the experimental control as a means of comparing an unaltered situation to one that was manipulated. He described over 180 different parasites, which included a number of arthropod ectoparasites. He correctly determined that many parasites grow up from eggs, making significant contributions to the germ theory of disease. In addition to all of his scientific contributions, Redi was an accomplished poet. His life-sized statue stands proudly looking down on all who visit the world-renowned Uffizi Museum in Florence, Italy.

36. *Paragonimus westermani*

(Kerbert 1878)

Pronunciation: \par-ə-'gän-ə-məs\ \west-ər-mən-ī\

Paragonimus kellicotti

(Ward 1908)

Pronunciation: \par-ə-'gän-ə-məs\ \kel-i-kot-ī\

Introduction

More than 40 species are in the genus *Paragonimus* (\par-ah-GON-i-mus\). Only nine are responsible for the majority of cases in humans: *P. westermani*, *P. africanus*, *P. heterotremus*, *P. kellicotti* (\KEL-ee-KOT-ee\), *P. mexicanus*, *P. siamensis*, *P. skrjabini*, *P. miyazakii*, and *P. uterobilateralis*.¹⁻¹⁰ An estimated 20 million people are infected with *Paragonimus* spp. Worldwide.¹¹ The most commonly reported trematode in this genus to cause human disease is *P. westermani*.



Figure 36.1. Adult of *Paragonimus westermani*. 10mm x 5 mm.

While most members of this genus are relatively restricted in distribution, *P. westermani* is widely found worldwide.¹¹ *P. westermani* will be presented as the model organism for food-borne lung flukes affecting humans. *P. kellicotti*, indigenous to the United States, and certain *Paragonimus* spp., will also be mentioned.¹²⁻¹⁴

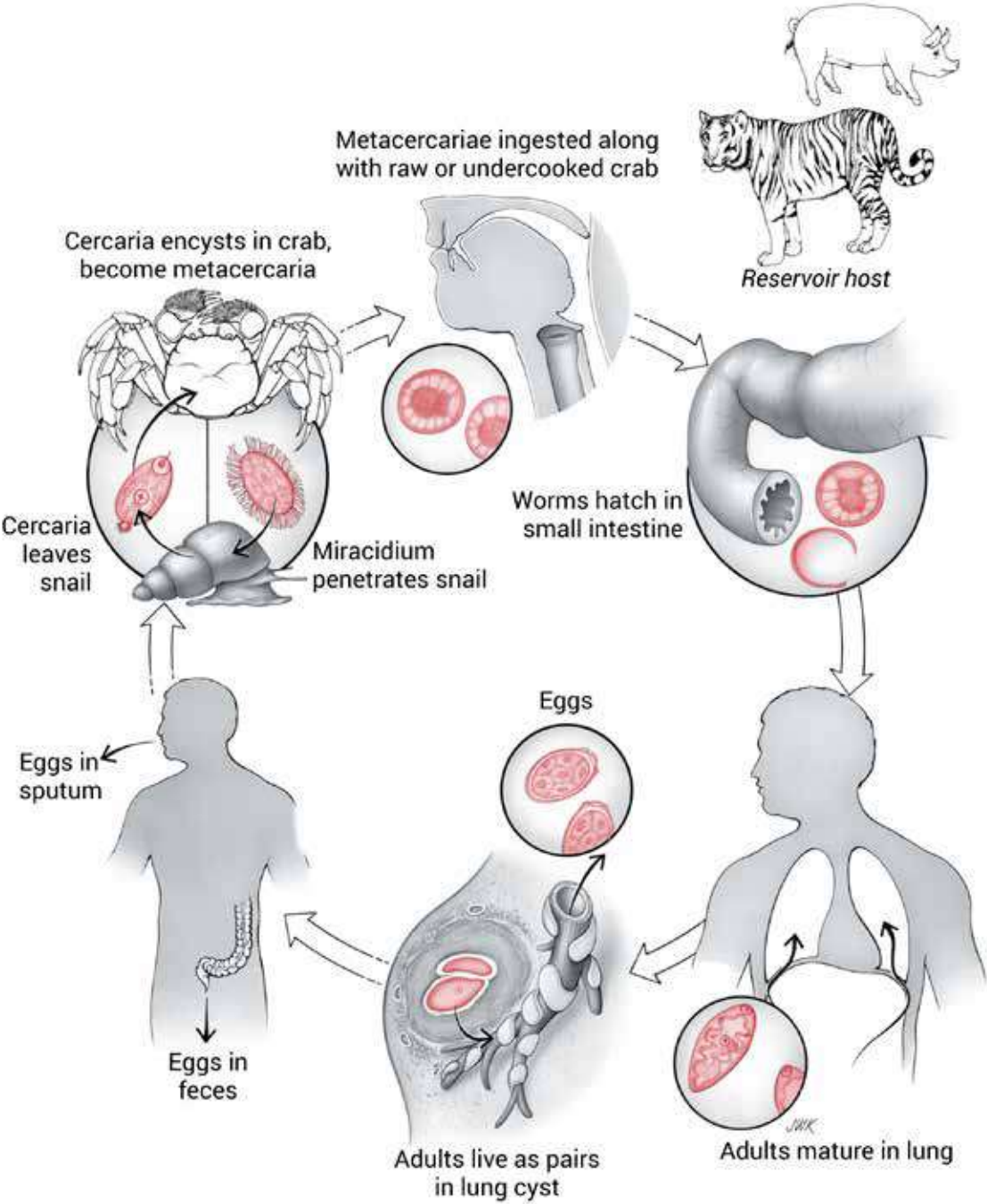
Although hermaphroditic, most *Paragonimus* spp. do not self-fertilize. Instead, they live typically as two or more worms in cysts or cavities, usually in lung tissue.¹³ Paragonimiasis occurs throughout Japan, China, Korea, Vietnam, Thailand, Cambodia, India, Micronesia, Indonesia, Papua New Guinea, and the Philippines.¹⁵⁻¹⁷ *P. westermani* infects many reservoir hosts, including fox, civet, tiger, leopard, panther, mongoose, wolf, pig, dog, and cat. It employs numerous crustaceans as intermediate hosts, accounting for its global distribution.¹¹

The genus *Paragonimus* is diverse.^{5, 18} Several other species routinely infect humans: *P. skrjabini* and *P. miyazakii* in Japan, *P. africanus* in Cameroon, *P. uterobilateralis* in Liberia and Nigeria, and *P. mexicanus* and *P. ecuadoriensis* in Latin America.⁵ *P. kellicotti*, a lung fluke of mink and opossums in the United States, has also caused human infection.¹²

Historical Information

1878 Coenraad Kerbert described the adult worm he isolated from a Bengal tiger at autopsy.¹⁹ In 1916, Koan Nakagawa implicated the freshwater crab as the intermediate host in the transmission of *P. westermani*.²⁰ In 1915, Sadamu Yokogawa deciphered the correct migration route of the immature adult fluke in the mammalian host.²¹ In 1880, one year after the first human case was described in an individual living in Taiwan, Erwin von Baelz and Patrick Manson reported on

Paragonimus westermani



most of the clinical features of the disease. Also, they identified eggs of *P. westermani* in the sputum of patients with hemoptysis.²² In 1899, Max Braun established the genus *Paragonimus*, with the name derived from the Greek words “para” (on the side of) and gonimos” (genitalia).²⁴

Life Cycle

The adult of *P. westermani* is large, measuring 10–12 mm by 5–7 mm (Fig. 36.1). It induces a fibrotic capsule of tissue at the periphery of the lung and lives there, usually as two or more worms. More than 50 species of crustaceans can support the next stage of the life cycle, with freshwater crabs (e.g., *Eriocheir* spp., *Potamon* spp., *Potamiscus* spp.) as the most common intermediate hosts throughout most of the Far East.¹³ In many Asian countries, crabs are eaten Raw or undercooked. In the U.S., *P. kellicotti* infection results from eating uncooked crayfish.²⁵

Infection begins by ingesting the metacercariae (Fig. 36.2), which excyst in the small intestine.¹³ Metacercariae penetrate the abdominal cavity and, within several days, develop into immature flukes. The worms

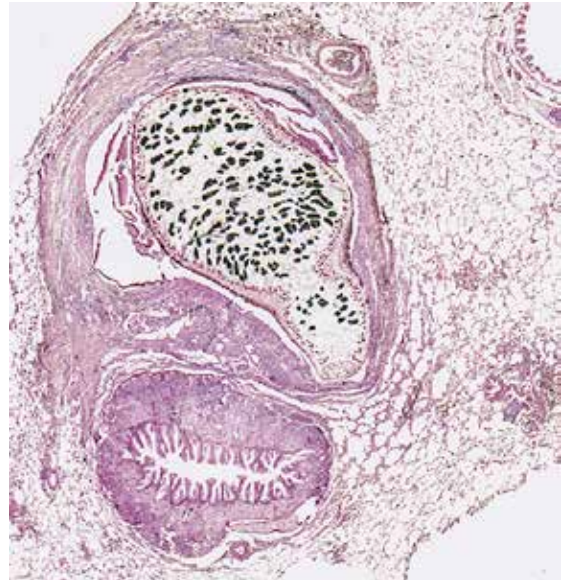


Figure 36.3. Histological section of adult *P. westermani* in lung.

migrate to the lungs by penetrating the diaphragm and mature into reproductive adults within 8–12 weeks (Fig. 36.3). Worms also locate aberrant sites, including the brain, liver, intestines, muscle, skin, and testes. In these sites, the passage of eggs to the external environment is impossible.

The pair of adults usually cross-fertilize each other. Both diploid and triploid forms of the adult *P. westermani* exist.¹⁸ The triploid form produces eggs via parthenogenesis. Egg pro-

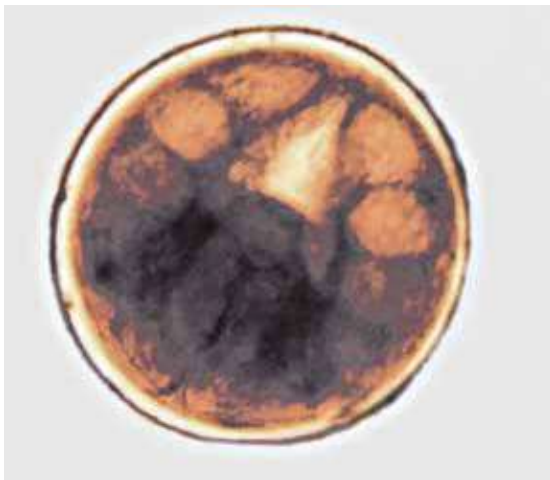


Figure 36.2. Metacercaria of *P. westermani*. 34 μ m.



Figure 36.4. Egg of *P. westermani*. 110 μ m x 60 μ m.

duction begins about 30 days after ingestion of the metacercariae. Eggs (Fig. 36.4) pass fertilized but unembryonated into the surrounding tissue. Eventually, they reach the bronchioles and are included in the sputum, which also contains blood and debris from the necrotic lesions created by the adults. Because some sputum is swallowed, eggs can be recovered from feces and sputum. The eggs must reach freshwater to embryonate. The miracidium develops over three weeks, after which it hatches and seeks out its intermediate snail host (e.g., *Melania* spp., *Semio-sulcospira* spp., and *Thiara* spp.). In contrast to other trematodes such as *Schistosoma* spp., *Paragonimus* spp. can mature in many snail species.¹³ *Paragonimus* spp. develop through the sporocyst and redia stages into cercariae, which then exit from the snail and encyst on and within crustacean intermediate hosts. In the case of the crab, the metacercariae infect all organs.

Cellular and Molecular Pathogenesis

Immature worms of *P. westermani* do not cause clinical disease, either on their way from the small intestine to the abdominal cavity or during the last leg of their journey to the lung tissue, except in the case of heavy infection.²⁶⁻²⁹ In contrast, mature adult worms in the lung form cysts that eventually communicate with the bronchioles.²⁴

Triploid forms of the parasite are considered more pathogenic than diploid forms. The diploid forms are smaller and will form cysts only if sexual partners are found.²⁴

Various immune cells characterize the inflammatory responses to *Paragonimus* cysts, but eosinophils typically predominate. Charcot-Leyden crystals can frequently be found in the sputum of infected individuals. Specific IgG and IgE antibodies are pro-

duced throughout the infection but appear to have no protective function.³⁰ Several stages of *P. westermani* secrete cysteine proteases that cleave IgG molecules, and the worm may employ this strategy to avoid immune damage by the host.³¹ Infections last somewhat longer than a year, after which adult worms die and become calcified.

Larval stages of several zoonotic species of *Paragonimus*, such as *P. skrjabini* and *P. miyazakii*, can cause extensive damage to the tissues as they migrate throughout the viscera.²⁴

Clinical Disease

The clinical manifestations of paragonimiasis include acute early infection and late or chronic disease. Early infection occurs between the ingestion of infective metacercariae and lasts until flukes mature into egg-producing adults. Some patients may remain asymptomatic during this stage of infection, while others present with diarrhea, fever, chest pain, fatigue, urticaria, epigastric pain, and eosinophilia.²⁶⁻²⁹ Patients may go on to develop cough with blood-tinged sputum, dyspnea, increasing leukocytosis and eosinophilia, and transient pulmonary infiltrates. In some situations, patients may present with cutaneous manifestations, noting painless migratory subcutaneous swellings.³² These subcutaneous nodules contain juvenile flukes.²⁴

Mature lung flukes trigger late-stage infection. Cough and recurrent hemoptysis are the most common clinical features.^{33, 34} Patients may also present with chest pain, dyspnea, fever, or chills.¹³ Depending on the severity of the infection and the frequency of bacterial superinfections, pneumothorax and pleural effusion may occur, resulting in pleural adhesions.

An often fatal clinical outcome results from extrapulmonary paragonimiasis. Immature flukes may migrate to several tissues, including the brain. Cerebral paragonimiasis is thought to be rare, estimated to occur in less than 1% of symptomatic individuals infected with *P. westermani*, but is associated with a significantly higher mortality rate than pulmonary disease.³⁵ Cerebral paragonimiasis has been observed with other *Paragonimus* spp, such as *P. kellicotti*.^{36, 37}

Diagnosis (see Clinical Appendix)

Diagnostic modality is based on the stage of the disease. Late-stage disease is diagnosed by microscopic identification of eggs in the sputum, bronchoalveolar lavage fluid, and more rarely in stool.^{38, 39} Several cases of acid-fast bacilli-negative pulmonary infections are due to paragonimiasis. One distinguishing histopathological feature is the presence of Charcot-Leyden crystals.⁴⁰ Eggs can be visualized microscopically using wet preps. For many years, acid-fast staining of sputum specimens destroyed *Paragonimus* eggs. This issue has been overcome using modern Ziehl-Neelsen staining, and using a 10x rather than the standard 100x objective employed in diagnosing mycobacterial infections.^{38, 39}

If sputum or other body fluids, such as CSF or pleural fluid, are negative for eggs on repeated sampling, indirect evidence of infection can be obtained using serological tests, such as ELISA and Western blot.⁴¹⁻⁴³ These tests are beneficial in early stage and extrapulmonary diseases. Immunoblot testing is available from the Centers for Disease Control (CDC), and ELISA tests are available from commercial laboratories.^{13, 27} Serology is also used in the diagnosis of *P. Ellicott* infection in the U.S.²⁵

Antigen detection tests have been developed but are only sometimes used in clinical practice.⁴⁴ NAATs are being developed to allow for species identification.⁴⁵ A simple and rapid intradermal test is performed by injecting diluted *Paragonimus* antigen into the skin.²⁴ Over 2 million people have been skin tested in China, with an overall positivity rate of 20%.²⁴ The serologic and intradermal assays indicate current or past exposure to the infection.

Imaging tests such as CT and fluorodeoxyglucose-positron emission tomography (FDG-PET) can help diagnose but are not definitive.⁴⁶⁻⁴⁸ In many cases, migratory tracks caused by helminth invasion can be visualized through and between the pleura and lung parenchyma.⁴⁹ Clinical diagnosis depends on suspicion of paragonimiasis in any patient from an endemic area with the characteristic pulmonary disease. Pulmonary paragonimiasis must be distinguished from chronic bronchiectasis, lung abscess due to other causes, and tuberculosis.^{16, 50}

Cerebral paragonimiasis must be distinguished from brain tumors and lesions caused by other helminths (e.g., juvenile tapeworms and *Fasciola hepatica*). The subcutaneous nodules of *P. skrjabini* must be differentiated from other forms of cutaneous larva migrans and gnathostomiasis.²⁴

Treatment (see Clinical Appendix)

Praziquantel is the drug of choice against *Paragonimus* spp., while triclabendazole is an alternative drug with similar efficacy.^{24, 50-54} Another regimen used is multiple rounds of praziquantel alternating with albendazole.⁵⁵ The complications of pleural effusions and subsequent fibrosis may sometimes require surgical management, including decortication.¹³

Prevention and Control

Because of its numerous reservoir hosts and worldwide distribution, many consider control of this parasite in animals impractical in most parts of the world.¹³ Due to cultural eating habits favoring the acquisition of this parasite, control of paragonimus infection is complex, and would require a comprehensive approach.⁵⁶ For example, “drunken hairy crab” is traditionally eaten live, and in the modern city of Shanghai, it is considered *haute cuisine*. Treatment of infected individuals, sanitation changes, and behavioral changes in handling and cooking intermediate crustacean hosts all have a role to play in

controlling paragonimiasis.

Mass chemotherapy has been attempted to reduce prevalence rates in certain endemic regions of the world.⁵⁷⁻⁶⁰ Boiling the invertebrate host for several minutes until the meat has turned opaque can kill the metacercariae. Marinating and salting of crabs or other crustaceans does not reliably kill the infective stages.²⁴ The overall burden of disease appears to be declining with improved sanitation and other factors, but diagnostic suspicion needs to be maintained since the re-emergence of this organism is always a possibility due to fluctuations of risk and relaxed control efforts.⁶¹

References

1. Iwagami, M., et al., Ancient divergence of *Paragonimus westermani* in Sri Lanka. *Parasitol Res* **2008**, *102* (5), 845-52.
2. Iwagami, M., et al., The first intermediate host of *Paragonimus westermani* in Sri Lanka. *Acta Trop* **2009**, *109* (1), 27-9.
3. Iwagami, M., et al., Identities of two *Paragonimus* species from Sri Lanka inferred from molecular sequences. *J Helminthol* **2003**, *77* (3), 239-45.
4. Iwagami, M., et al., Molecular phylogeographic studies on *Paragonimus westermani* in Asia. *J Helminthol* **2000**, *74* (4), 315-22.
5. Iwagami, M., et al., A molecular phylogeographic study based on DNA sequences from individual metacercariae of *Paragonimus mexicanus* from Guatemala and Ecuador. *Journal of helminthology* **2003**, *77* (1), 33-8.
6. Iwagami, M., et al., A molecular phylogeographic study based on DNA sequences from individual metacercariae of *Paragonimus mexicanus* from Guatemala and Ecuador. *J Helminthol* **2003**, *77* (1), 33-8.
7. Agatsuma, T., et al., The origin of the triploid in *Paragonimus westermani* on the basis of variable regions in the mitochondrial DNA. *J Helminthol* **2003**, *77* (4), 279-85.
8. Blair, D., et al., *Paragonimus skrjabini* Chen, 1959 (Digenea: Paragonimidae) and related species in eastern Asia: a combined molecular and morphological approach to identification and taxonomy. *Syst Parasitol* **2005**, *60* (1), 1-21.
9. Doanh, P. N., et al., *Paragonimus* and paragonimiasis in Vietnam: an update. *Korean J Parasitol* **2013**, *51* (6), 621-7.
10. Chai, J. Y., Paragonimiasis. *Handb Clin Neurol* **2013**, *114*, 283-96.
11. Furst, T., et al., Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. *Lancet Infect Dis* **2012**, *12* (3), 210-21.
12. Mariano, E. G., et al., A human infection with *Paragonimus kellicotti* (lung fluke) in the United States. *American journal of clinical pathology* **1986**, *86* (5), 685-7.
13. Procop, G. W., North American paragonimiasis (Caused by *Paragonimus kellicotti*) in the context of global paragonimiasis. *Clin Microbiol Rev* **2009**, *22* (3), 415-46.
14. Diaz, J. H., Paragonimiasis acquired in the United States: native and nonnative species. *Clin Microbiol Rev* **2013**, *26* (3), 493-504.
15. Kim, E.-A., et al., Imaging findings of hepatic paragonimiasis : a case report. *Journal of Korean*

- medical science* **2004**, 19 (5), 759-62.
16. Singh, T. N., et al., Pulmonary paragonimiasis. *The Indian journal of chest diseases & allied sciences* **2004**, 46 (3), 225-7.
 17. Owen, I. L., Parasitic zoonoses in Papua New Guinea. *Journal of helminthology* **2005**, 79 (1), 1-14.
 18. Blair, D., Genomes of *Paragonimus westermani* and related species: current state of knowledge. *International journal for parasitology* **2000**, 30 (4), 421-6.
 19. Kerbert, C., Zur Trematodenkenntnis. *Zool Anz* **1878**, 1, 271-273.
 20. Nakagawa, K.; J., The mode of infection in pulmonary distomiasis: certain fresh water crabs as intermediate hosts of *Paragonimus westermani*. *Dis* **1916**, 18, 131-142.
 21. Yokogawa, S., On the route of migration of *Paragonimus westermani* in the definitive host. *aiwan Igakkai Zasshi* **1915**, 152, 685-700.
 22. Von Baelz, E. O. E., Uber Parasitare Haemoptoe (gregarinosi pulmonum). *Zentralbl Med Wissenschr* **1880**, 18 721-722.
 23. Manson, P., *Distoma ringeri*: Medical Report for the Half Year Ended 30 September. 1880; Vol. 2, p 10-12.
 24. Blair, D., et al., Paragonimiasis and the genus *Paragonimus*. *Advances in parasitology* **1999**, 42, 113-222.
 25. Fischer, P. U.; Weil, G. J., North American paragonimiasis: epidemiology and diagnostic strategies. *Expert Rev Anti Infect Ther* **2015**, 13 (6), 779-86.
 26. Zhong, H. L., et al., Recent progress in studies of *Paragonimus* and paragonimiasis control in China. *Chin Med J (Engl)* **1981**, 94 (8), 483-94.
 27. Kagawa, F. T., Pulmonary paragonimiasis. *Semin Respir Infect* **1997**, 12 (2), 149-58.
 28. DeFraín, M.; Hooker, R., North American paragonimiasis: case report of a severe clinical infection. *Chest* **2002**, 121 (4), 1368-72.
 29. Uchiyama, F., et al., Re-emergence of paragonimiasis in Kyushu, Japan. *Southeast Asian J Trop Med Public Health* **1999**, 30 (4), 686-91.
 30. Kong, Y., et al., Immunoglobulin G (IgG) subclass and IgE responses in human paragonimiasis caused by three different species. *Clinical and diagnostic laboratory immunology* **1998**, 5 (4), 474-8.
 31. Chung, Y. B., et al., Activities of different cysteine proteases of *Paragonimus westermani* in cleaving human IgG. *The Korean journal of parasitology* **1997**, 35 (2), 139-42.
 32. Hatano, Y., et al., Expression of Th1 and Th2 cytokine mRNAs in freshly isolated peripheral blood mononuclear cells of a patient with cutaneous paragonimiasis. *J Dermatol Sci* **1999**, 19 (2), 144-7.
 33. Singh, T. S., et al., Pulmonary paragonimiasis: clinical features, diagnosis and treatment of 39 cases in Manipur. *Trans R Soc Trop Med Hyg* **1986**, 80 (6), 967-71.
 34. Im, J. G., et al., Pleuropulmonary paragonimiasis: radiologic findings in 71 patients. *AJR Am J Roentgenol* **1992**, 159 (1), 39-43.
 35. Singh, T. S., et al., Cerebral paragonimiasis mimicking tuberculoma: First case report in India. *Trop Parasitol* **2011**, 1 (1), 39-41.
 36. Centers for Disease, C.; Prevention, Human paragonimiasis after eating raw or undercooked crayfish --- Missouri, July 2006-September 2010. *MMWR Morb Mortal Wkly Rep* **2010**, 59 (48), 1573-6.
 37. Lane, M. A., et al., Human paragonimiasis in North America following ingestion of raw crayfish. *Clin Infect Dis* **2009**, 49 (6), e55-61.
 38. Barennes, H., et al., Paragonimiasis as an important alternative misdiagnosed disease for suspected acid-fast bacilli sputum smear-negative tuberculosis. *Am J Trop Med Hyg* **2014**, 90 (2), 384-5.
 39. Slesak, G., et al., Ziehl-Neelsen staining technique can diagnose paragonimiasis. *PLoS Negl Trop Dis* **2011**, 5 (5), e1048.
 40. Luo, J., et al., Pulmonary Paragonimiasis Mimicking Tuberculous Pleuritis: A Case Report. *Medicine (Baltimore)* **2016**, 95 (15), e3436.
 41. Ikeda, T., Cystatin capture enzyme-linked immunosorbent assay for immunodiagnosis of human paragonimiasis and fascioliasis. *The American journal of tropical medicine and hygiene* **1998**, 59 (2), 286-90.
 42. Slemenda, S. B., et al., Diagnosis of paragonimiasis by immunoblot. *The American journal of*

- tropical medicine and hygiene* **1988**, 39 (5), 469-71.
43. Dekumyoy, P., et al., Human lung fluke *Paragonimus heterotremus*: differential diagnosis between *Paragonimus heterotremus* and *Paragonimus westermani* infections by EITB. *Tropical medicine & international health : TM & IH* **1998**, 3 (1), 52-6.
 44. Zhang, Z., et al., Antigen detection assay to monitor the efficacy of praziquantel for treatment of *Paragonimus westermani* infections. *Trans R Soc Trop Med Hyg* **1996**, 90 (1), 43.
 45. Intapan, P. M., et al., Genomic characterization of lung flukes, *Paragonimus heterotremus*, *P. siamensis*, *P. harinasutai*, *P. westermani* and *P. bangkokensis* by RAPD markers. *Vet Parasitol* **2004**, 124 (1-2), 55-64.
 46. Lee, I. J., et al., Organizing pneumonia by paragonimiasis and coexistent aspergilloma manifested as a pulmonary irregular nodule. *Case Rep Radiol* **2011**, 2011, 692405.
 47. Kim, K. U., et al., A pulmonary paragonimiasis case mimicking metastatic pulmonary tumor. *Korean J Parasitol* **2011**, 49 (1), 69-72.
 48. Song, J. U., et al., Pulmonary paragonimiasis mimicking lung cancer in a tertiary referral centre in Korea. *Int J Tuberc Lung Dis* **2011**, 15 (5), 674-9.
 49. Akaba, T., et al., Pulmonary Paragonimiasis: The Detection of a Worm Migration Track as a Diagnostic Clue for Uncertain Eosinophilic Pleural Effusion. *Intern Med* **2016**, 55 (5), 503-6.
 50. Johnson, R. J.; Johnson, J. R., Paragonimiasis in Indochinese refugees. Roentgenographic findings with clinical correlations. *The American review of respiratory disease* **1983**, 128 (3), 534-8.
 51. Johnson, R. J., et al., Paragonimiasis: diagnosis and the use of praziquantel in treatment. *Reviews of infectious diseases* **1985**, 7 (2), 200-6.
 52. Udonsi, J. K., Clinical field trials of praziquantel in pulmonary paragonimiasis due to *Paragonimus uterobilateralis* in endemic populations of the Igwun Basin, Nigeria. *Trop Med Parasitol* **1989**, 40 (1), 65-8.
 53. Ripert, C., et al., Therapeutic effect of triclabendazole in patients with paragonimiasis in Cameroon: a pilot study. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1992**, 86 (4), 417.
 54. Keiser, J.; Utzinger, J., Chemotherapy for major food-borne trematodes: a review. *Expert opinion on pharmacotherapy* **2004**, 5 (8), 1711-26.
 55. Hu, Y., et al., Pleuropulmonary paragonimiasis with migrated lesions cured by multiple therapies. *Indian J Pathol Microbiol* **2016**, 59 (1), 56-8.
 56. Sharma, O. P., The man who loved drunken crabs. A case of pulmonary paragonimiasis. *Chest* **1989**, 95 (3), 670-2.
 57. Kim, J. S.; Bang, F. B., [A Follow-Up Study To Evaluate The Efficacy Of Mass Chemotherapy For Control Of Paragonimiasis]. *Kisaengchunghak Chapchi* **1974**, 12 (1), 8-13.
 58. Kim, J. S., Mass Chemotherapy In The Control Of Paragonimiasis. *Kisaengchunghak Chapchi* **1969**, 7 (1), 6-14.
 59. Xu, Z. B., Studies on clinical manifestations, diagnosis and control of paragonimiasis in China. *Southeast Asian J Trop Med Public Health* **1991**, 22 Suppl, 345-8.
 60. Choi, D. W., *Paragonimus* and paragonimiasis in Korea. *Kisaengchunghak Chapchi* **1990**, 28 Suppl, 79-102.
 61. Blair, D., Lung flukes of the genus *Paragonimus*: ancient and re-emerging pathogens. *Parasitology* **2022**, 149 (10), 1286-1295.

37. Other Trematodes of Medical Importance

Several other trematode species deserve mention, but not to the extent of the schistosomes or more prevalent food-borne trematodes. A few of these rare infections are endemic in some geographic regions and deserve more than a mention since exotic infections are becoming more common in high-income country health facilities due to increased immigration from endemic areas. Many species are zoonotic and classified as emerging infections in some locales.¹ They include at least 59 different species of intestinal flukes found in Southeast Asia.²

Fasciolopsis buski (Lankaster 1857)

Pronunciation: \ˈfä-shōˈläp-səs\bu-skē\

Three trematodes in the Fasciolidae family are reported to infect humans: *Fasciola hepatica*, *F. gigantica*, and *Fasciolopsis buski* (FAH-see-ow-lop-sis\Boo-skee).³ *F. hepatica* is considered a trematode of paramount human importance (Chapter 35). *F. gigantica* is very similar in biology and geography to *F. hepatica* and will not be discussed further in this section. *Fasciolopsis buski*, the giant intestinal fluke, is a large trematode (Fig. 37.1), similar in morphology to *F. hepatica*. *F. buski* is the most common intestinal trematode infection in humans and lives attached to the columnar epithelium of the small intestine. Infection occurs in China, Taiwan, Vietnam, Thailand, Bangladesh, and India (including the Bihar State).^{4,7} Reservoir hosts include dogs and rabbits.

Life Cycle

The infectious stage for mammals is the metacercaria, which is found on the husks of the seeds of littoral freshwater plants (e.g., lotus, water chestnut, water caltrop and other com-

mercial crops in which human feces is used as fertilizer). Once eaten, the metacercaria excysts in the small intestine and attaches to the luminal surface.

The adult matures within 2–4 months and measures 20–30 mm by 10 mm. In contrast to the longer life spans of other trematodes, *F. buski* only lives for about one year. After self-fertilization, egg-laying begins. The large, oval, unembryonated eggs (Fig. 37.2) are passed out with the fecal mass. If they reach warm (i.e., 25–30 °C) freshwater, they immediately undergo embryogenesis and hatch within 5–8 weeks. The miracidium that emerges penetrates a snail (e.g., *Segmentina* spp. and *Heppentis* spp.) and develops sequentially first into sporocysts, then rediae, and finally into cercariae. After leaving the snail, the cercariae swim about and rest on lit-

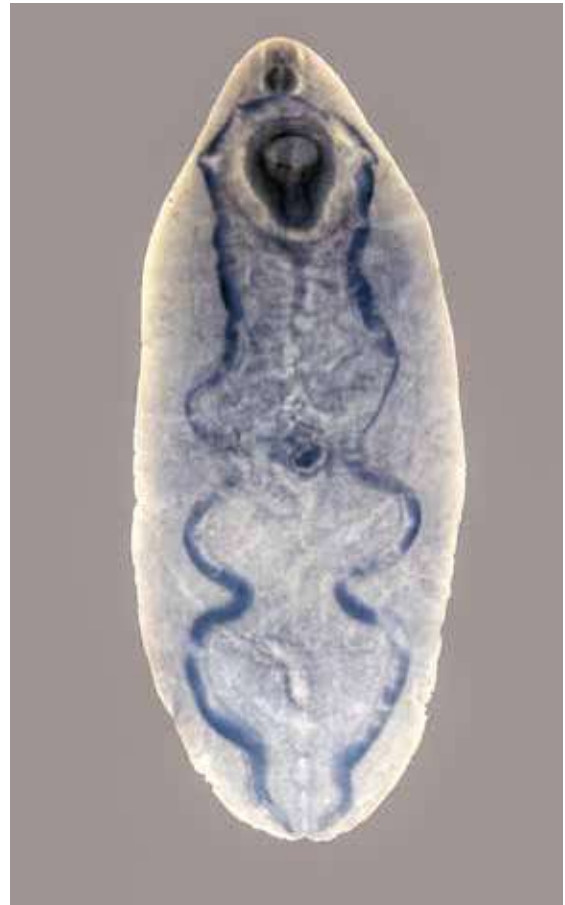
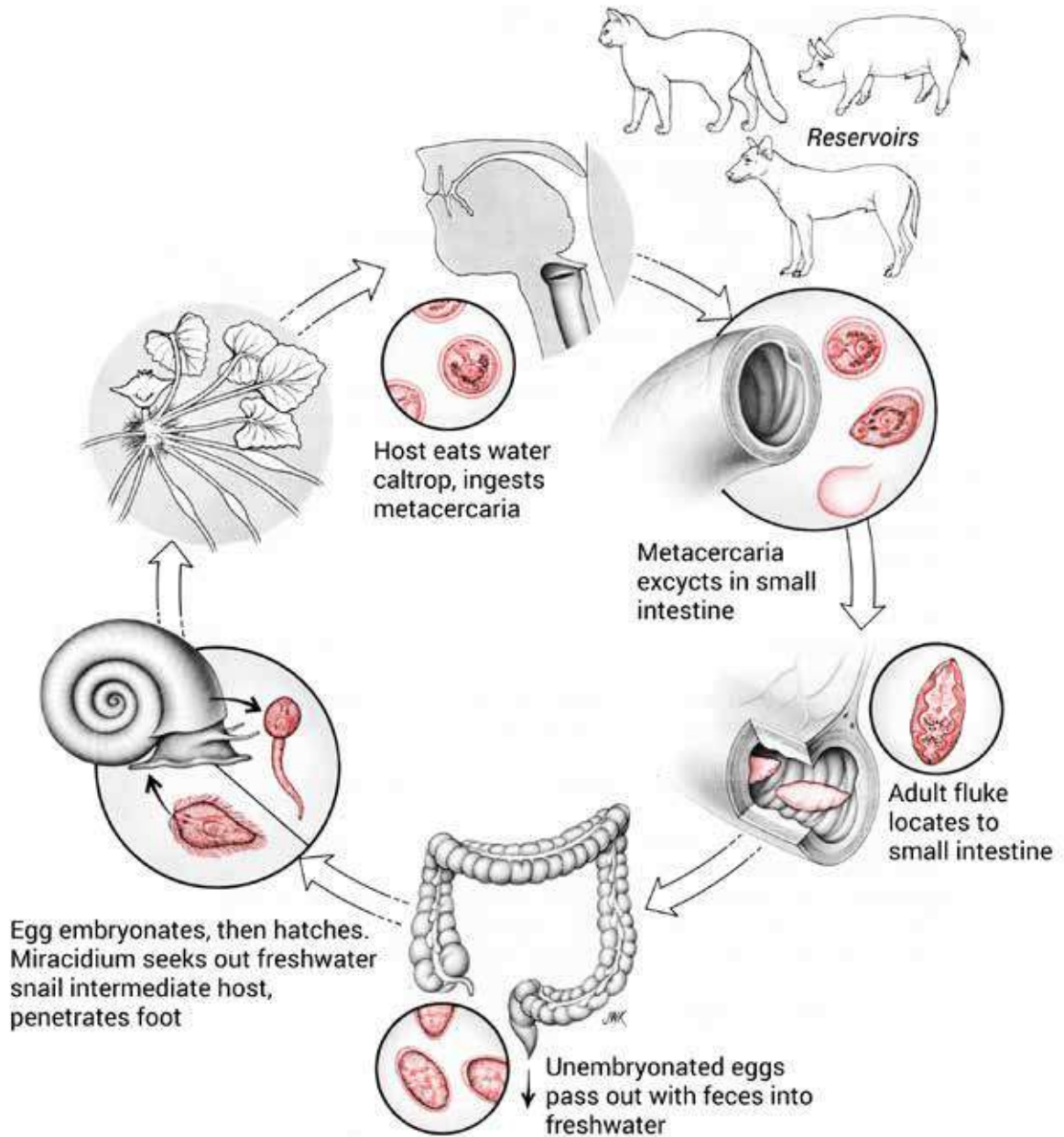


Figure 37.1. Adult of *Fasciolopsis buski*. 25 mm x 10mm.

Fasciolopsis buski

toral vegetation. The metacercaria develops and then encysts there, awaiting ingestion by an unsuspecting host.

Clinical Disease

The worm feeds on columnar epithelial cells, injuring the tissue. Light infection does not cause clinical disease, although intermittent diarrhea may result. Heavy infection (i.e., hundreds of worms) produces continuous diarrhea, nausea, vomiting, fever, intestinal bleeding, obstruction of the ampulla of Vater, blockage of the common bile duct, and, in extreme cases, blockage of the small intestine. Abdominal pain is a common complaint, simulating the signs and symptoms of peptic ulcer. Allergic-type reactions with swelling of the legs and face have been reported. Hypoproteinemia, vomiting, anemia, and weight loss have been described in heavily-infected children.⁸ Elevated circulating eosinophilia is a common feature of even light infection with *F. buski*.

Diagnosis

Definitive diagnosis is the microscopic identification of the egg or flukes in stool or vomit.⁹

¹⁰

Treatment

The drug of choice is praziquantel.^{11, 12}

Prevention and Control

Proper disposal of human feces is the primary method of control. Travelers to endemic areas should ensure that all aquatic plants are well-cooked before ingestion. Reservoir hosts play a role in the maintenance of this parasite in endemic regions, and consequently, it is recommended that raw aquatic plants are not fed to pigs.¹³ Shucking water chestnuts by plac-

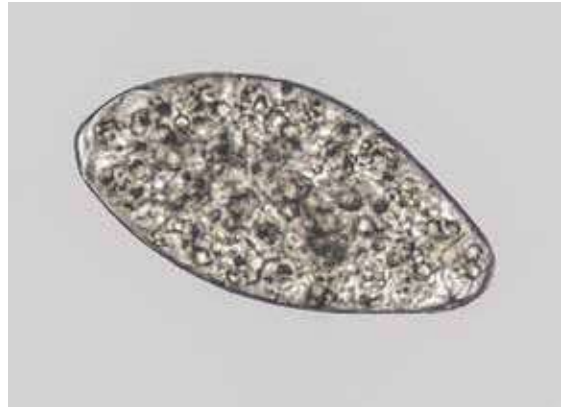


Figure 37.2. Egg of *F. buski*. 140 μm x 80 μm .

ing the seed pod in one's mouth and biting through the tough outer husk provides an infection route. This activity is still common in some areas, but public health education programs have helped reduce infection.⁶

Echinostoma spp.

Pronunciation: \i-ˌkī-nə-ˈstō-mə\

The genus *Echinostoma* (eh-KYE-no-STO-mah) has at least 24 species, with 15 capable of infecting humans.^{1, 14} These trematodes are found throughout Southeast Asia, with endemic foci in Korea, China, India, Indonesia, Thailand, the Philippines, and Malaysia.¹⁴⁻¹⁶ Their life cycles are similar to Fasciola's, except that the metacercariae encyst in various snails, tadpoles, or freshwater fish species.¹⁷

Adults live in the small intestine, and the symptoms they induce depend on the degree of infection. Diarrhea, nausea, vomiting, and abdominal pain are commonly experienced, usually accompanied by fever.

Diagnosis is made through the microscopic identification of the characteristic eggs in stool or, in some cases, through the identification of the flukes on endoscopy. Currently, the recommended treatment is with single-

dose praziquantel, but other antihelmintic therapies may have some degree of efficacy.

¹⁴

Heterophyes heterophyes

(Siebold 1852)

Pronunciation: \he-tə- ,rō'fī-ēz\\he-tə- ,rō'fī-ēz\\

Metagonimus yokogawai

(Katsurada 1912)

Pronunciation: \me-tə-gōn-ə-məs\\yō-kō-gu-wī\\

Heterophyes heterophyes ((\HET-er-OW-fye-eez\\HET-er-OW-fye-eez\\) (Fig. 37.3) and *Metagonimus yokogawai* (\META-gone-u-mus\\yoo-koo-guh-why\\ (Fig. 37.4) are small flukes that live primarily in the small intestine. They cause minor damage there. *H. heterophyes* is found throughout Asia, the Middle East, and Africa. *M. Yokogawa* is also common in Asia, but foci of infections have



Figure 37.3. Adult of *Heterophyes heterophyes*. 2 mm x 0.5 mm.



Figure 37.4. Egg of *H. heterophyes*. 25 μ m x 13 μ m.

been reported in Spain and Russia. A few human infections with *Heterophyes nocens*, a related species, have been reported from Korea.¹⁹

Life Cycles

Infection begins with ingesting encysted metacercariae that live just under the skin of certain freshwater fishes (e.g., grass carp).²⁰ The metacercariae excyst in the small intestine and develop into adult worms.

Although a rare event, instead of remaining in the small intestine, adult worms can migrate to other organs, such as the heart or brain, where they cause focal granulomas with variable clinical consequences. Both species of trematodes self-fertilize, and egg production ensues shortly after that. The fully-embryonated eggs pass out with the fecal mass into brackish or freshwater.

H. heterophils primarily infect snails of the genus *Cerithidia*, while those of *M. Yokogawa* infect snails in the genera *Semisulcospira* and *Thiara*. The embryonated eggs are ingested by their respective snail hosts and hatch inside, releasing the miracidia. This stage undergoes sequential development in the snail, first to sporocysts, then to rediae, and finally to cercariae. The cercariae penetrate out of the snail

and, like those of *Clonorchis sinensis*, encyst under the skin of freshwater fish or in frogs, tadpoles, or even another snail.¹⁴ The species of intermediate Hosts for both parasites vary widely depending on the geographic locale. In Asia, the intermediate hosts are cyprinoid and salmonid fishes, and in the Middle East, mullet and tilapia are primarily involved with the life cycle.

Clinical Disease

Like other trematode infections, the clinical presentation is thought to be determined in



Figure 37.5. An adult of *Metagonimus yokogawai*. 2.5 mm x 0.6 mm.



Figure 37.6. Egg of *M. yokogawai*. 25 μ m x 15 μ m.

large part by the worm burden.²¹ Epigastric distress, fatigue, diarrhea, weight loss, and malaise have been reported for heavy infection, along with belching, headache, nausea, vomiting, and even urinary incontinence.^{19, 22}

Diagnosis (see Clinical Appendix)

Diagnosis is usually based on recovery and identification of eggs in feces. The eggs (Figs. 37.5, 37.6) of *H. heterophyes* and *M. yokogawai* closely resemble those of *C. sinensis* (Fig. 34.4). They must be carefully differentiated by their absence of a terminal knob and a collar at the operculum. Eggs and even adult flukes have been recovered on endoscopy.^{18, 23,}

²⁴

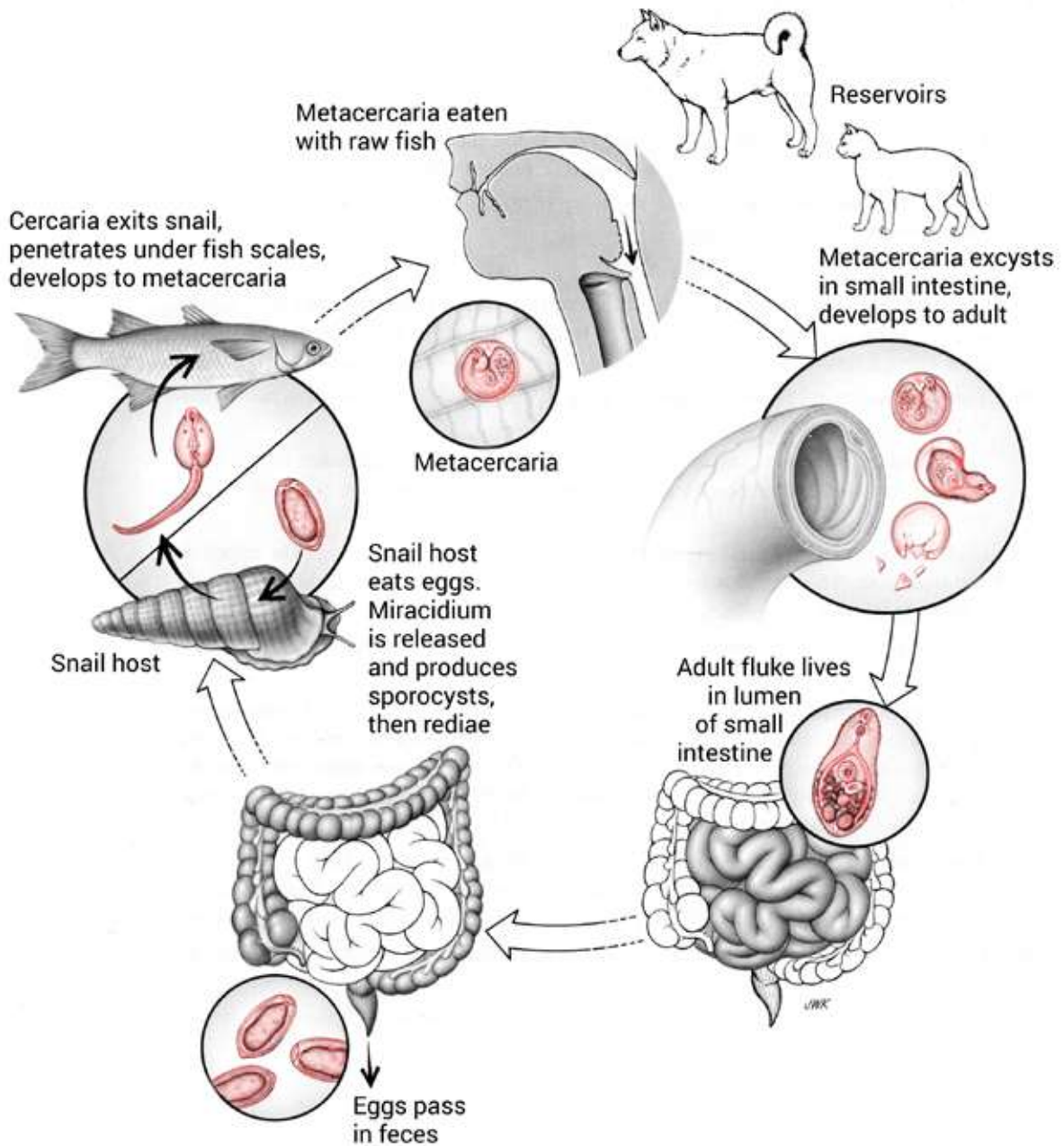
Treatment (see Clinical Appendix)

The drug of choice is praziquantel. Mebendazole may be an alternative but less efficacious option.²⁵

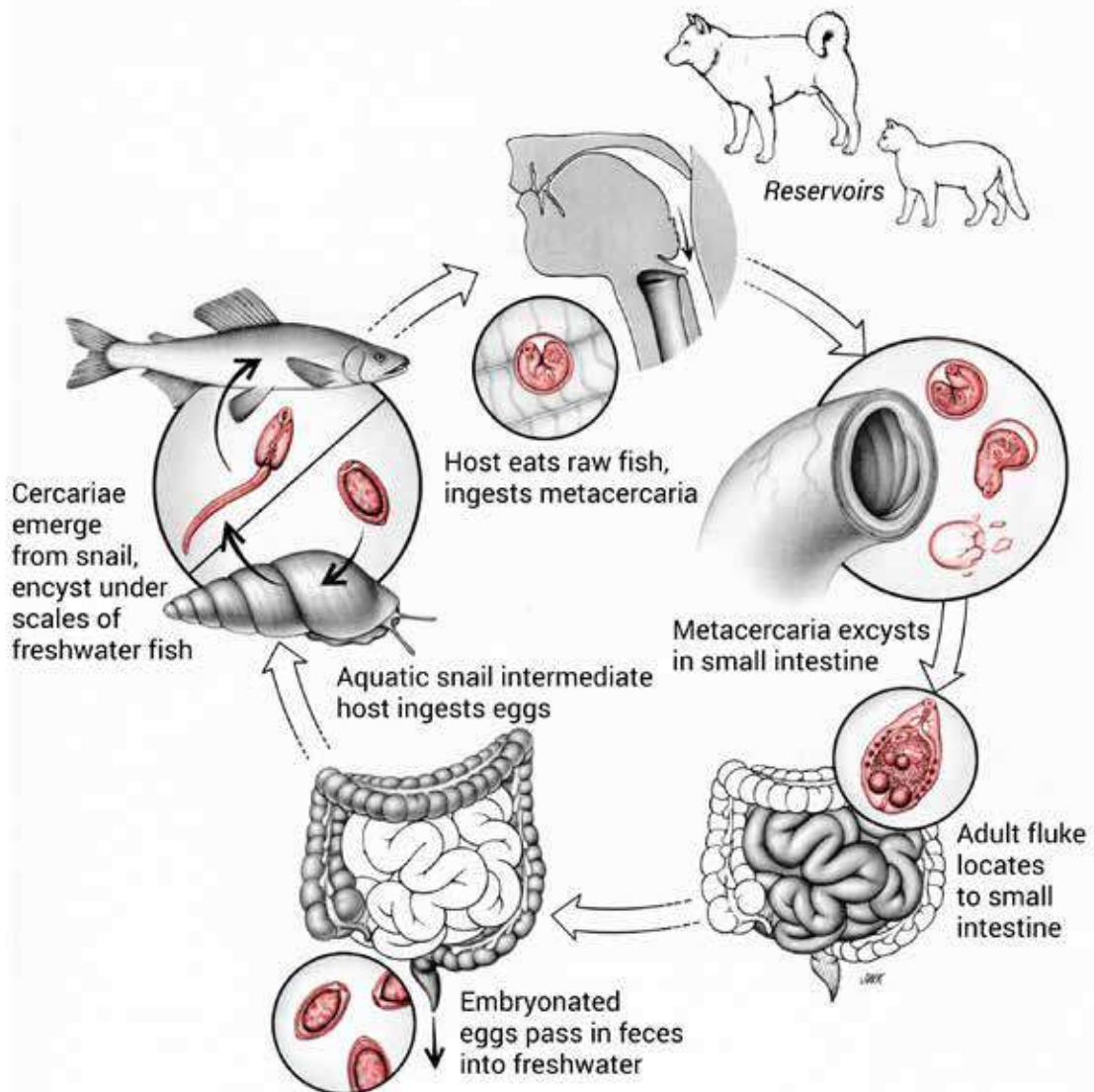
Prevention and Control

Heterophyiasis can be prevented by eating only cooked fish and eliminating the indiscriminate use of untreated human feces as fertilizer.^{1, 17, 20} Protecting fish ponds from contamination with human feces and controlling snail populations are potentially helpful.¹⁴

Heterophyes heterophyes



Metagonimus yokogawai



Nanophyetus salmincola

(Chapin 1927)

Pronunciation: \nānə, fī'ētəs\\sal-min-kō-lə\

Although *Nanophyetus salmincola* (\NAH-no-FIE-ee-tus\\SAL-min-koo-la\) may be the most common trematode in the United States, it mainly infects animals and rarely infects humans.²⁶ *N. salmincola* infects dogs, foxes, and coyotes in eastern Siberia and in the Pacific Northwest of the United States, where it produces “salmon poison-

ing” or “Elokomin fluke fever” as a result of a rickettsia, *Neorickettsia helmintheca*, which is co-transmitted with the parasite.²⁷ Human infection has also been described, resulting in diarrhea, nausea, vomiting, cachexia, anorexia, and elevated levels of circulating eosinophils.²⁸ *N. salmincola* infection is diagnosed by the presence of characteristic eggs in the stools and a history of ingesting raw or poorly cooked salmon.²⁸ Praziquantel is the recommended treatment, but niclosamide may also be efficacious.²⁸

References

1. Fried, B., et al., Food-borne intestinal trematodiasis in humans. *Parasitology research* **2004**, 93 (2), 159-70.
2. Chai, J. Y., et al., Foodborne intestinal flukes in Southeast Asia. *Korean J Parasitol* **2009**, 47 Suppl, S69-102.
3. Keiser, J.; Utzinger, J., Food-borne trematodiasis. *Clin Microbiol Rev* **2009**, 22 (3), 466-83.
4. Wiwanitkit, V.; Suyaphan, A., High prevalence of HBsAg seropositivity in Hilltribers in the Mae Jam district in northern Thailand. *MedGenMed : Medscape general medicine* **2002**, 4 (3), 26.
5. Bhatti, H. S., et al., Fasciolopsiasis--a re-emerging infection in Azamgarh (Uttar Pradesh). *Indian journal of pathology & microbiology* **2000**, 43 (1), 73-6.
6. Graczyk, T. K., et al., Fasciolopsiasis: is it a controllable food-borne disease? *Parasitology research* **2001**, 87 (1), 80-3.
7. Achra, A., et al., Fasciolopsiasis: Endemic focus of a neglected parasitic disease in Bihar. *Indian J Med Microbiol* **2015**, 33 (3), 364-8.
8. Gupta, A., et al., *Fasciolopsis buski* (giant intestinal fluke)--a case report. *Indian journal of pathology & microbiology* **1999**, 42 (3), 359-60.
9. Naher, B. S., et al., Fasciolopsiasis in a five year old girl. *Mymensingh Med J* **2013**, 22 (2), 397-9.
10. Le, T. H., et al., Case report: unusual presentation of *Fasciolopsis buski* in a Vietnamese child. *Trans R Soc Trop Med Hyg* **2004**, 98 (3), 193-4.
11. Taraschewski, H., et al., Effects of praziquantel on human intestinal flukes (*Fasciolopsis buski* and *Heterophyes heterophyes*). *Zentralblatt für Bakteriologie, Mikrobiologie, und Hygiene. Series A, Medical microbiology, infectious diseases, virology, parasitology* **1986**, 262 (4), 542-50.
12. Chai, J. Y., Praziquantel treatment in trematode and cestode infections: an update. *Infect Chemother* **2013**, 45 (1), 32-43.
13. Inpankaew, T., et al., A survey for potentially zoonotic gastrointestinal parasites of dogs and pigs in Cambodia. *Acta Parasitol* **2015**, 60 (4), 601-4.
14. Toledo, R.; Esteban, J. G., An update on human echinostomiasis. *Trans R Soc Trop Med Hyg* **2016**, 110 (1), 37-45.
15. Park, S. K., et al., Status of intestinal parasite infections among children in Bat Dambang, Cambodia. *The Korean journal of parasitology* **2004**, 42 (4), 201-3.
16. Huffman, J. E.; Fried, B., *Echinostoma* and echinostomiasis. *Advances in parasitology* **1990**, 29, 215-69.
17. Anantaphruti, M. T., Parasitic contaminants in food. *The Southeast Asian journal of tropical medicine and public health* **2001**, 32 Suppl 2, 218-28.
18. Jung, W. T., et al., A case of *Echinostoma cinetorchis* (Trematoda: Echinostomatidae) infection

- diagnosed by colonoscopy. *Korean J Parasitol* **2014**, 52 (3), 287-90.
19. Chai, J.-Y., et al., Prevalence of *Heterophyes nocens* and *Pygydiopsis summa* infections among residents of the western and southern coastal islands of the Republic of Korea. *The American journal of tropical medicine and hygiene* **2004**, 71 (5), 617-22.
20. Chai, J.-Y.; Lee, S.-H., Food-borne intestinal trematode infections in the Republic of Korea. *Parasitology international* **2002**, 51 (2), 129-54.
21. Graczyk, T. K.; Fried, B., Echinostomiasis: a common but forgotten food-borne disease. *Am J Trop Med Hyg* **1998**, 58 (4), 501-4.
22. Chang, Y. D., et al., A human infection of *Echinostoma hortense* in duodenal bulb diagnosed by endoscopy. *Korean J Parasitol* **2005**, 43 (2), 57-60.
23. Toledo, R., et al., Intestinal trematode infections. *Adv Exp Med Biol* **2014**, 766, 201-40.
24. Esteban, J. G., et al., Diagnosis of human trematode infections. *Adv Exp Med Biol* **2014**, 766, 293-327.
25. Chen, Y., et al., Studies on efficacy of praziquantel and mebendazole-medicated salt in treatment of *Echinochasmus fujianensis* infection. *Southeast Asian J Trop Med Public Health* **1997**, 28 (2), 344-6.
26. Eastburn, R. L., et al., Human intestinal infection with *Nanophyetus salmincola* from salmonid fishes. *Am J Trop Med Hyg* **1987**, 36 (3), 586-91.
27. Headley, S. A., et al., *Neorickettsia helminthoeca* and salmon poisoning disease: a review. *Vet J* **2011**, 187 (2), 165-73.
28. Eastburn, R. L., et al., Human intestinal infection with *Nanophyetus salmincola* from salmonid fishes. *The American journal of tropical medicine and hygiene* **1987**, 36 (3), 586-91.



Ronald Ross, M.D. (1857–1932)

Ross, working in India as a member of the British Medical Service, followed advice from Patrick Manson and decided to work on malaria. Ross eventually succeeded in making the connection between mosquitoes and the transmission of malaria, using birds as hosts and culicine mosquitoes as the arthropod vector in his experiments. When Ross then tried to show the same was also true for the transmission of human malaria, he failed, since only anopheline mosquitoes are vectors for all of the human malarias. Ross was gifted in mathematics and developed epidemiological models for describing malaria epidemics. His models are still in use today.

Giovanni Grassi and colleagues in Italy, at around the same time that Ross published his original findings, carried out the seminal work on human malaria transmission. Eventually, Ross was awarded the Nobel Prize in 1902 for his discovery. However, controversy surrounds that event, because Grassi claimed (and perhaps rightly so), that his work was far more significant than Ross's, and therefore he, not Ross, should have received the prize. It has been suggested that Grassi did not receive full credit because of a conflict Grassi had with Robert Koch when Koch came to work in Grassi's laboratory. Koch served on the Nobel Prize Committee when the 1902 prize selection occurred and chose to ignore Grassi's findings and honor Ross instead.

VIII. The Arthropods

Arthropods directly influence humans' well-being, not only because they are hosts of parasitic organisms and vectors of a wide variety of pathogens, but also by causing tissue damage and disease. They also affect human health by reducing the availability of food. Insects are essential pollinators of crops, but they also destroy an estimated 20% of all food crops. This destruction continues despite the increasing use of pest management tactics such as insecticides, genetically modified organisms and physical control methods in fields and storage areas. Livestock are also affected by arthropod-borne infections. Vast areas of Africa are short of protein foods because cattle suffer a number of vector-borne diseases, including trypanosomiasis transmitted by tsetse flies and a variety of tick-transmitted diseases.

Although the pathogenic effects of arthropods are most pronounced in the tropics, they are by no means negligible in the United States and other temperate areas. Lyme disease and anaplasmosis, which are transmitted by ticks, have spread rapidly throughout the United States. *Aedes albopictus*, the Asian tiger mosquito, has been introduced in shipments of used automobile tires into the southern United States and has spread as far north as central Ohio, Indiana, and Illinois; moreover, the introduced strain of the mosquito is apparently able to survive the winter in the egg stage in temperate climates. The same species has been introduced into Europe and South America. *Ae. albopictus* can be an efficient vector of dengue, chikungunya and Zika virus. In 1999, reports surfaced about the introduction and rapid spread across the Eastern United States of another pest mosquito, *Ochlerotatus japonicus*. Introductions of new species should not be unexpected and point to the ease with which such introductions can occur. The unexpected appearance in New

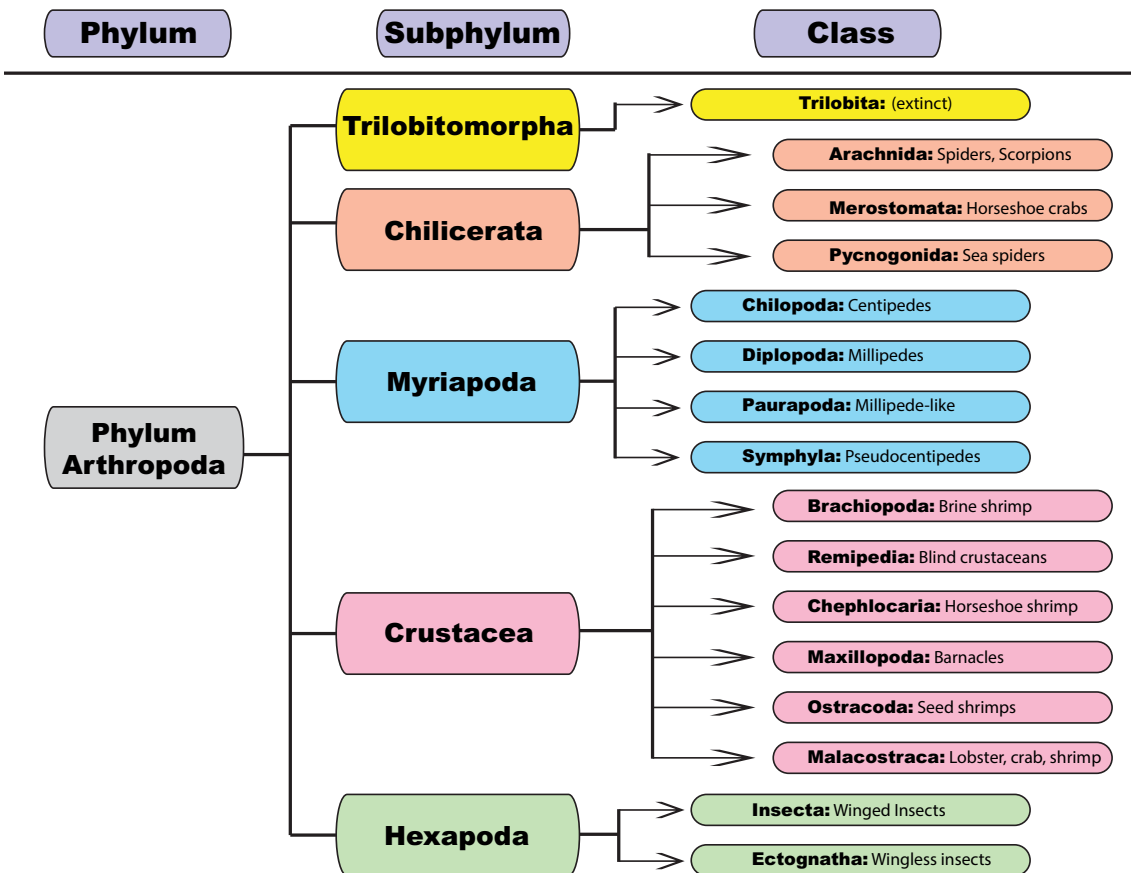
York City in 1999 of mosquito-transmitted human infections of the West Nile virus, and its subsequent spread throughout the United States, should reinforce our awareness of vulnerability to invasion by both pathogens and vectors. Although fear has been expressed that blood-feeding arthropods, especially mosquitoes, could transmit HIV, a large body of epidemiological and experimental evidence fails to support this hypothesis.

Despite the problems arthropods cause for humans and livestock, they are also beneficial as pollinators, producers of honey, natural regulators of harmful insects, and essential members of food chains. The phylum Arthropoda contains an enormous diversity of members, with the number of species exceeding that of all other phyla combined. The arthropods share a number of characteristics that distinguish them from all other animal groups, although some of these features are absent in a particular species or group at some period of development. Nevertheless, there are traits specific to each class in the phylum to help identify them.

Among the morphologic characteristics are bilateral symmetry, a hard exoskeleton, a segmented body, and paired, jointed appendages. The term arthropod, derived from Greek, means "jointed foot." Growth by metamorphosis is another characteristic of the arthropods. In some groups, growth is gradual; each change from one stage to the next is known as a molt, and gives rise to a stage somewhat larger but morphologically similar to its predecessor (incomplete metamorphosis). Among the spiders, eight or nine immature stages may precede the final molt to the sexually mature adult. Another developmental strategy involves egg, larva, pupa and adult. In this case, each stage is morphologically distinct (complete metamorphosis). Examples include the flies and the fleas.

The application of the tools of molecular biology to the study of arthropods is pervasive. Most dramatic are the various genome projects. The genome of *Anopheles gambiae*, the most important of the African malaria vectors, is complete, and work on several other vectors is either completed or in process. The genomic sequences of *Aedes aegypti*, the Yellow Fever mosquito, *Culex pipiens* the

vector of West Nile Virus and *Ixodes scapularis*, the primary vector of Lyme disease in the U.S. have been completed. Mitochondrial genomes for the sand flies *Phlebotomus papatasi* and *P. chinesis* have been reported. The value of these programs and their use in eventual control of the diseases these vectors transmit remains to be determined.



38. Insects

Introduction

The insects have two distinct types of development. The more primitive insect orders pass through a series of stages by incomplete metamorphosis (Fig. 38.1). A typical life cycle involves the egg, a (usually) fixed number of immature nymph stages, and the mature adult stage. The insect molts between stages, sheds its old exoskeleton, and reveals a new skin within. Nymphs are similar to the adult, but lack wings and are sexually immature.

In contrast, complete metamorphosis is characteristic of some of the more advanced insect orders, including the flies (Diptera; Fig. 38.2) and the fleas (Siphonaptera; Fig 38.3). The life cycle of an insect exhibiting complete metamorphosis includes the egg, larval stages, a pupa stage and the adult stage.

Table 38.1 lists arthropods of importance to human health, the pathogens they harbor and transmit, and the diseases they cause. The methods by which the arthropod vectors transmit pathogens vary. Some pathogens, unchanged by any interaction with the vector, are transmitted mechanically from one host to another on contaminated legs or mouthparts of the arthropod or in its feces. Other pathogens require passage through the arthropod as part of their life cycle. In such cases, the pathogens undergo specific developmental changes, which usually include multiplication within the arthropod.

Arthropods can also be pathogens themselves. They can infest the host, migrating through the body or developing *in situ* while feeding on host tissue. Other arthropods cause mechanical injury through bites, chemical injury through injection of venom, or allergic reactions to the materials they transmit via the bite or sting. Moreover, entomopho-

bia and arachnophobia (i.e., fear of insects and arachnids, particularly spiders) are not uncommon psychological conditions and are distinct from delusional parasitosis (Ekbom's syndrome) where an individual becomes convinced they are infested with parasites when they are not.¹⁻³

The salivary secretions of arthropods in general, and insects in particular, have proven to be extraordinarily complex. These secretions serve as potent immunogens and stimulate the bothersome allergic reactions to the insect's bite. They also serve, in many cases, to carry the viral, bacterial, protozoal or nematode pathogens for which so many arthropods serve as vectors. These salivary secretions evolved not to cause allergic responses or convey pathogens, but for a much more basic reason. They facilitate the capacity of the arthropod to take blood from a host whose physiology and defense mechanisms are designed to prevent the loss of blood.

In almost all blood-sucking arthropods studied to date, the saliva of each species has at least one anticlotting, one vasodilator and one antiplatelet compound. The molecular diversity of these compounds is great, even among closely related genera of blood feeders.^{4,5}

Diptera: The Flies

No single group of insects has so affected human evolution, development, or history as the Diptera, the order of insects comprised of flies and mosquitoes. Malaria, yellow fever, elephantiasis, sleeping sickness, dengue, and river blindness are among the more serious diseases carried by members of this large order. Notorious as vectors of pathogenic organisms of humans and animals, dipterans are also important for the mechanical damage (i.e., myiasis) caused by their larvae and the allergic responses caused by the bites of some adults.

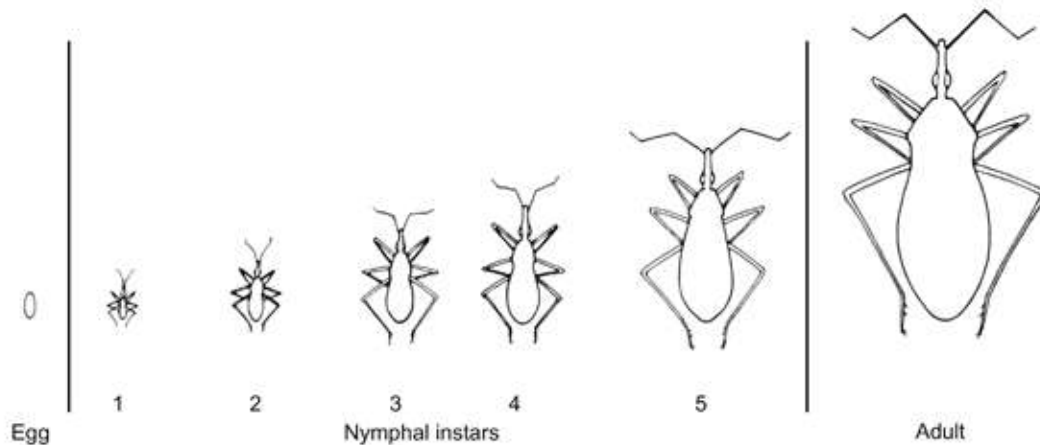


Figure 38.1. Incomplete metamorphosis in insects. Typical example of incomplete metamorphosis is the kissing bug, *Rhodnius prolixus*. Immature stages are wingless, smaller versions of the winged adult. All stages, except the egg, have three pairs of legs. The number of nymphal stages varies with the species.

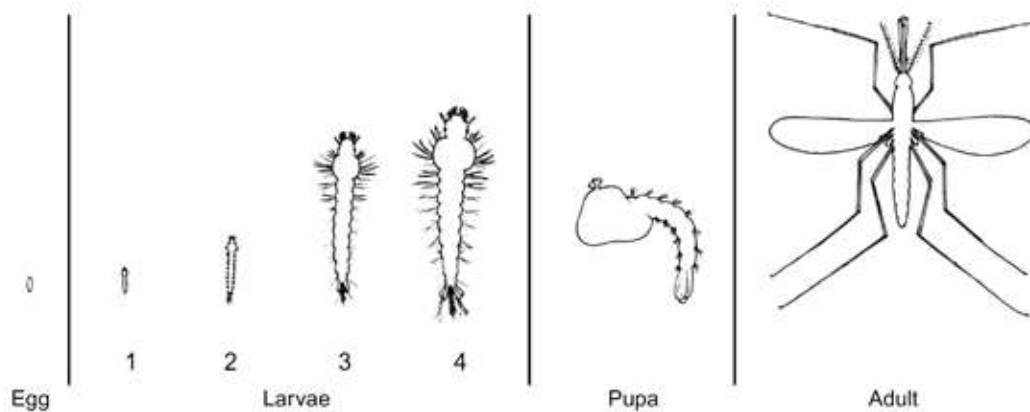


Figure 38.2. Complete metamorphosis in an insect with aquatic immature stages. The *Anopheles* mosquito begins as an egg laid on the surface of water and develops through four larval stages and a single pupal stage to a sexually mature, winged adult.

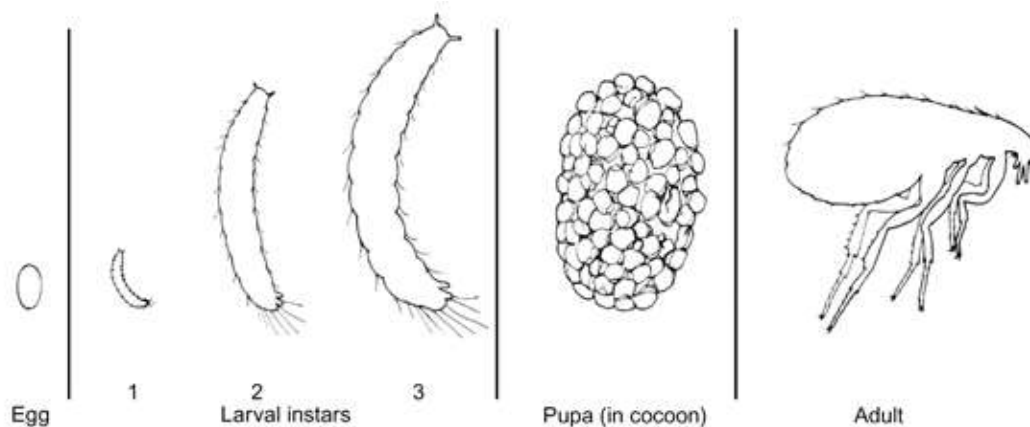


Figure 38.3. Complete metamorphosis in an insect with terrestrial immature stages. The flea begins as an egg laid on the fur of the host or in the nest area. Several maggot-like larval stages are followed by a single pupal stage, encased in a sand-covered cocoon, from which a wingless, sexually mature adult flea emerges.

Table 38.1. Arthropods of medical importance.

Order and representative species Insecta	Common name	Geographic distribution	Effects on humans
Anoplura (sucking lice)			
<i>Pediculus humanus humanus</i>	Body louse	Worldwide	Skin reactions to bites, vectors of rickettsiae and spirochetes
<i>P. humanus capitis</i>	Head louse	Worldwide	Skin reaction to bites
<i>Phthirus pubis</i>	Crab louse	Worldwide	Skin reaction to bites
Heteroptera (true bugs)			
<i>Cimex lectularius</i>	Bed bug	Worldwide	Skin reaction to bites
<i>C. hemipterus</i>	Tropical bed bug	Tropical and subtropical	Skin reaction to bites
<i>Triatoma infestans</i> <i>Rhodnius prolixus</i> <i>Panstrongylus megistus</i>	Kissing bug, cone-nosed bug	Tropical and subtropical regions of the New World	Skin reaction to bites, vectors of <i>Trypanosoma cruzi</i> (cause of Chagas disease)
Hymenoptera (bees, wasps, ants)			
<i>Apis mellifera</i>	Honey bee	Worldwide	Painful sting, potential anaphylaxis
<i>Bombus</i> spp.	Bumble bee	Worldwide	Painful sting, potential anaphylaxis
Various genera and species of the family Vespidae	Wasp, hornet, yellow jacket	Worldwide	Painful sting, potential anaphylaxis
<i>Solenopsis</i> spp.	Fire ant	Tropical America, southeastern United States	Painful bite and multiple stings, potential anaphylaxis
Diptera (flies, mosquitoes, and their relatives)			
Ceratopogonidae <i>Culicoides</i> spp. <i>Leptoconops</i> spp.	No-see-um, sand fly	Worldwide	Serious biting pest, skin reaction to bites, vectors of several filariid nematodes
Psychodidae <i>Phlebotomus</i> spp. <i>Lutzomyia</i> spp.	sand fly	Worldwide	Skin reaction to bites, vectors of Leishmania, Pappataci fever, and Carrion's disease
Simuliidae <i>Simulium</i> spp.	Black fly, buffalo gnat	Worldwide	Serious biting pests, skin reaction to bites, vectors of Onchocerca and Mansonella
Culicidae <i>Aedes</i> spp. <i>Anopheles</i> spp. <i>Culex</i> spp. <i>Culiseta</i> spp. <i>Mansonia</i> spp.	Mosquito	Worldwide	Serious biting pests, skin reaction to bites, vectors of viruses, protozoa, and filaria
Tabanidae <i>Tabanus</i> spp.	Horsefly	Worldwide	Biting pests, painful bite followed by skin reaction
<i>Chrysops</i> spp.	Deerfly	Worldwide	Biting pests, vectors of tularemia and <i>Loa loa</i> .
Muscidae <i>Musca domestica</i> <i>Stomoxys calcitrans</i>	House fly Stable fly	Worldwide Worldwide	Mechanical disseminator of pathogens Serious biting pest
Glossinidae <i>Glossina</i> spp.	Tsetse fly	Africa	Vector of trypanosomes of humans and animals
Calliphoridae Cuterebridae Sarcophagidae Larvae of various genera and species	Maggots	Worldwide	Myiasis, accidental or obligate development of larval flies in human tissue
Siphonaptera (fleas)			
<i>Xenopsylla cheopis</i> <i>Ctenocephalides felis</i> <i>C. canis</i> <i>Pulex irritans</i> <i>Tunga penetrans</i>	Oriental rat flea Cat flea Dog flea Human flea Chigoe flea	Worldwide Worldwide Worldwide Worldwide Africa and South America	Vector of plague Biting pest Biting pest Biting pest, plague vector Infestation of toes, feet and legs, causing severe pain and secondary infection

Flies develop by complete metamorphosis and have distinct larval, pupal, and adult forms. Larvae are usually vermiform, often living in water or damp places or developing in living or dead tissue. Pupae represent a non-feeding transitional stage. Adult dipterans, which usually possess wings, have only one pair (*diptera* means “two wings”). The mouthparts of the adults may be adapted for biting, piercing flesh, and sucking blood, or only for sponging fluids.

Diptera is a large order, divided into three suborders containing over 100 families. Only nine families are of medical concern. The most primitive suborder, the Nematocera, contains four medically important families: Ceratopogonidae, Psychodidae, Simuliidae, and Culicidae. In the suborder Brachycera, only the Tabanidae are of any medical significance; in the third suborder, Cyclorrhapha, the Muscidae, Gasterophilidae, Cuterebridae, and Oestridae are of major concern.

Ceratopogonidae: The Biting Midges

Ceratopogonids, commonly called punks, no-see-ums, sand flies, or midges, are minute (0.4–5.0 mm long), slender, blood-sucking dipterans. They constitute a serious pest problem in many areas of the tropics, the temperate zones, and in the Arctic. Most of the species that affect humans belong to two genera, *Culicoides* and *Leptoconops*, which act as vectors of several filariids that infect humans in Africa and the New World. Species of *Culicoides* may also serve as vectors of filariids and viruses that infect animals, including humans.

Life Cycle

Ceratopogonid larvae develop in aquatic or

semi-aquatic habitats, often in freshwater, but usually in brackish water or tidal flats. Some important species are associated with the highly polluted runoff from livestock holding areas. Larval stages are long, slender, and snake-like; in some species they undergo diapause, a state of arrested development, for as long as three years while awaiting optimal environmental conditions. Adult female ceratopogonids require blood for egg production. They typically feed at dusk and may attack in large numbers.

Pathogenesis

The mouthparts of ceratopogonids are short and lancet-like, producing a painful bite. Because of their large numbers, they can be important pests, particularly in beach and resort areas near salt marshes. Bites can produce local lesions that persist for hours or days. Sensitized individuals develop allergic reactions.

Midges of the genus *Culicoides* are the main vectors of the filariid nematodes: *Mansonella perstans* and *M. streptocerca* in Africa, and *M. ozzardi* in the New World tropics.

Control

Ceratopogonids develop in a wide range of habitats, and each species presents its own special problems with respect to control. Salt marsh habitats can be drained, or channeled, and other breeding sites modified. Treatment of breeding sites with insecticides remains the most effective short-term control measure. Window screens are ineffective unless they are treated with insecticides, because they allow these minute insects easy entry. Commercial mosquito repellents containing DEET may be useful against some of the common species of these pests.

Psychodidae: Moth Flies or Sand Flies

A single subfamily, the Phlebotominae, contains members that suck blood. Phlebotomine sand flies are small (1–3 mm), hairy, delicate, weak-flying insects that feed on a wide range of cold- and warm-blooded animals, and transmit a number of viral, bacterial, and protozoan infections. Flies of the genera *Phlebotomus* and *Lutzomyia* are important as vectors of the leishmaniae.

Historical Information

In 1921, Edouard and Etienne Sargent demonstrated the role of phlebotomine flies in the transmission of leishmaniasis.⁶

Life Cycle

Phlebotomine larvae develop in non-aquatic habitats such as moist soil, animal burrows, termite nests, loose masonry, stonewalls, or rubbish heaps. The four larval stages are completed within 2–6 weeks; the pupa stage may last 8–14 days. The adults (Fig. 38.4) are weak fliers, exhibiting a hopping movement rather than sustained flight. Only female phlebotomine sand flies require blood, feeding usually at night. Some species prefer to feed on humans, but none are exclusively



Figure 38.4. Adult female phlebotomine sand fly, *Lutzomyia anthophora*. Courtesy J. Ribeiro.

anthropophilic; they feed on dogs and rodents as well.

Pathogenesis

The mouthparts of the female phlebotomine are short and adapted for piercing and sucking. The bite may be painful, producing an itchy local lesion. Sensitized individuals may show severe allergic reactions. The saliva of the sand fly is particularly complex and contains a number of potent compounds that can influence the susceptibility of human macrophages to invasion by promastigotes of *Leishmania* introduced by the feeding vector. The use of components of sand fly saliva as a vaccine to prevent infection is being actively pursued and has received encouraging preliminary results.^{7,8}

Although phlebotomines cause problems in some areas as pests, they are of particular concern as vectors of a number of diseases. Bartonellosis (also called Carrion's disease, Oroya fever, or *verruca peruana*) is a South American disease transmitted from human to human by *Lutzomyia verrucarum* and related species. It is caused by the bacterium *Bartonella bacilliformis*, which invades erythrocytes and reticuloendothelial cells. The organism can produce a severe febrile illness, complicated by profound anemia. Untreated, bartonellosis is fatal in 50% of cases. There is no known animal reservoir.

Sand fly fever, also called papatasi fever, is a viral disease seen in the Mediterranean region, central Asia, Sri Lanka, India, and China. *Phlebotomus papatasi* is the main vector for this acute febrile disease, which is characterized by severe frontal headaches, malaise, retro-orbital pain, anorexia, and nausea. Female flies become infected when they feed on viremic individuals. After an incubation period of 7–10 days, the flies become infective and remain so for the rest of their lives.

Leishmaniasis is transmitted by a number of phlebotomine species. Flies initially pick up the parasite while feeding on infected humans or animals. In the fly, the parasite undergoes asexual multiplication, eventually accumulating in the mouthparts, from where it is transmitted to the host when the insect feeds.⁹⁻¹¹

Control

An integrated pest management approach is recommended for control of *Psychodidae* and includes filling in potential breeding sites and moving manure away from dwellings. Phlebotomine sand flies are particularly sensitive to insecticides, and the use of DDT in campaigns against malaria coincidentally controlled these flies and virtually eliminated sand fly-borne diseases in many regions. In areas where malaria has been eliminated or where malaria control programs have been abandoned, the phlebotomine flies have reestablished themselves, and the sand fly-borne diseases have returned. Larvicide use is challenged by the difficulty of getting this to where these larval populations live.

Sand flies may be controlled with residual or short-lived insecticides applied to breeding sites or houses. Treatment of window screens with insecticides may also be effective. Mosquito repellents can be used to reduce the frequency of sand fly bites.

Simuliidae: Black Flies

Members of the family Simuliidae, commonly called black flies, buffalo gnats, or turkey gnats, are small (1–5 mm long), hump-backed, blood-sucking dipterans that usually breed in fast-flowing streams and rivers. Simuliids are important as vectors of *Onchocerca volvulus*, the causative agent of river blindness. In addition, they present a serious pest problem in many temperate and



Figure 38.5. Black fly adult feeding on a human host.

Arctic areas. Black flies may also serve as vectors of bovine onchocerciasis and protozoan parasites of various species of birds.

Historical information

Black flies first stimulated medical interest in 1910, when it was suggested, incorrectly, that these flies transmitted a malaria-like organism that caused pellagra.¹² Their role in the transmission of *Onchocerca* was demonstrated in 1926.

Life Cycle

Adult female simuliids (Fig. 38.5) lay eggs at or below the surface of moving, well-oxygenated water. Larvae and pupae, equipped with gills for respiration, remain attached to objects below the surface. Larvae are nourished by food filtered from the passing water. They undergo development in five stages. The non-feeding pupae gradually assume adult characteristics while they are enclosed within a cocoon. Adult female simuliids require blood for egg production. They feed primarily during daylight hours. Male simuliids do not feed on blood.

Adult black flies in temperate areas may emerge synchronously in large numbers. Their bites cause serious damage to humans and animals. Black flies often reach such high

population densities that they can kill livestock and wild animals, torment campers and fishermen, and render large areas uninhabitable by humans and animals for long periods of time.

Pathogenesis

The bite of the female simuliid is particularly painful. The insect's mouthparts consist of six blades in the shape of lancets, which tear the skin surface to induce bleeding. The fly feeds from the resulting pool of blood, and the bite wound continues to bleed for some time after the fly has departed. Simuliid bites leave a characteristic point of dried blood at the wound site. The extreme pain of the initial bite is followed by itching and swelling due to reactions to the injected salivary secretions. Blood loss from multiple bites can be considerable. Allergic reactions in previously sensitized individuals are common, and can sometimes reach serious levels, including anaphylaxis. Reports from the Midwestern U.S. in the early part of the 20th century mention human deaths due to swarms of biting black flies.

Control

Control of black flies is most effectively achieved by the slow dripping of insecticides into rivers or streams. Mass control programs using insecticides applied by fixed-wing aircraft and helicopters have been successful in West African onchocerciasis. *Bacillus thuringiensis israelensis* (Bti) is a type of bacteria used for control of this insect in many parts of the world, including the United States.¹³ Clearing debris from streambeds can also reduce breeding. Repellents containing DEET are recommended for personal protection.

Culicidae: The Mosquitoes

Mosquitoes, although not one of the largest

dipteran families, are of major significance as vectors of disease and as biting pests. Their economic, cultural, and evolutionary impact has been devastating. Mosquitoes develop in a wide range of aquatic larval habitats and in all climates from the Arctic to the tropics. Adult mosquitoes are generally similar in appearance. They are usually small and have delicate legs, a single pair of wings, long antennae, and elongated mouthparts capable of piercing flesh and siphoning blood as well as drawing nectar from flowers. Larvae and pupae are aquatic and their development proceeds through complete metamorphosis (Fig. 38.2).

Historical Information

Numerous writers had suggested the association between mosquitoes and various tropical fevers in the past. The association of these fevers with mosquitoes was finally recognized during the nineteenth century. In 1878, Patrick Manson was able to observe that mosquitoes that fed on human blood from individuals with elephantiasis would then have the same unsheathed microfilaria he had observed in the blood of the patients.¹⁴ He was thus observing that these mosquitoes were intermediate hosts of *Wuchereria bancrofti*. In 1899, Thomas Bancroft was able to demonstrate that the filarial parasite, *W. bancrofti*, could be transmitted back to humans from the bite of a mosquito.¹⁴ This was only 6 years after Theobald Smith and Frederick L. Kilbourne, first demonstrated that ticks could transmit disease.¹⁵

Transmission of malaria by mosquitoes was suggested by Manson as early as 1884, but Ronald Ross and Italian investigators under the direction of Giovanni Grassi were the first to prove that mosquitoes transmitted malaria.¹⁶⁻¹⁸ The incrimination of *Aedes aegypti* in the transmission of yellow fever was suggested by Carlos Finlay in 1880, and proved by Walter Reed and his co-workers in 1900.¹⁹

Life Cycle

Both major subfamilies of the Culicidae – the Anophelinae and the Culicinae – are involved in transmission of diseases. Members of these subfamilies share several basic similarities in their life cycles and development. They lay eggs on or near water or on surfaces that become flooded. Their larvae are always aquatic. The four larval stages are elongate, active “wigglers” that feed by filtering particulate matter from water; they must remain in contact with the surface for respiration. The pupae, known as “tumblers,” are comma-shaped and aquatic. They remain at the surface unless disturbed.

Adult mosquitoes of most species are good fliers. Males and females feed on nectars and sugars, although females of most species also feed on blood. They require a blood meal for each clutch of eggs, which may contain 100–200 eggs. A female may produce six or more clutches during her lifetime. Eggs require 48–72 hours to develop within the female. They may be deposited almost as soon as they mature. Consequently, a female may take a blood meal every 2–4 days and contact a number of hosts during that period, providing an excellent opportunity for the dissemination of pathogens.



Figure 38.6. *Anopheles dirus*, one of the major malaria vectors in Southeast Asia, performing “plasma-pheresis”.



Figure 38.7. *Anopheles stephensi*, a malaria vector found in Asia, particularly India and Pakistan.

Subfamily Anophelinae

The genus *Anopheles* contains the species responsible for the transmission of human malaria. The anopheline female lays eggs singly, each equipped with floats, usually on the surface of water. Eggs hatch 2–4 days after they are laid. The aquatic larvae attach to the surface and assume a horizontal position. The larval period may last 1–3 weeks, depending on temperature. Anopheline pupae are superficially similar to the pupae of other mosquitoes. The pupal stage lasts 1–3 days. Adult anophelines (Fig. 38.6) are delicate, long-legged mosquitoes. Although some species are capable of extended flight and dispersion from breeding sites, anophelines typically remain close to their food supplies and breeding habitats.



Figure 38.8. *Anopheles freeborni*, a potential malaria vector in California.

Most anophelines are night feeders, with characteristic peaks of biting activity for each species. Some species are exclusively zoophilic, some are anthropophilic, and others are nonspecific biters. Feeding habits also vary between species. Certain species readily enter houses and feed on sleeping individuals; others feed only outdoors.

In temperate areas, anophelines spend the winter as inseminated adult females. In the tropics, these mosquitoes breed continually, although their population levels may fluctuate drastically in relation to rainfall and dry seasons.

Approximately 300 species of *Anopheles* mosquitoes have been described. However, only a small number of species are important as malaria vectors within any geographic area (e.g., *An. gambiae* and *An. funestus* in Sub-Saharan Africa, *An. culicifacies* and *An. stephensi* (Fig. 38.7) on the Indian subcontinent, and *An. quadrimaculatus* and *An. freeborni* (Fig. 38.8) in North America). Populations vary within each species with respect to their competence as vectors and capacity for transmission.

Intense study has led to the division of several well-established species of vectors into morphologically similar but genetically distinct groups or complexes of species. The important vector of malaria in Africa, *An. gambiae*, consists of at least six discrete but cryptic species, most of which are not major vectors. Similar revision of species has resulted in a clearer definition of the members of the European complex and the Southeast Asian group (Fig. 38.6). It appears that reexamination of most of the anopheline species that occupy large or ecologically diverse geographic areas will lead to the description of closely related but genetically divergent species.

Anophelines also play an important role as vectors of filarial nematodes. *An. gambiae* and *An. funestus* are the main vectors of *Wuchereria bancrofti* in Africa, and *An. hyrcanus* in China and *An. barbirostris* in Southeast Asia are vectors of both *W. bancrofti* and *Brugia malayi*. Although anophelines are not usually involved in the transmission of viruses, *An. gambiae* and *An. funestus* are the vectors of O'nyong-nyong fever.

Subfamily Culicinae

The subfamily Culicinae consists of more than 1500 species distributed among 20 genera, six of which (*Aedes*, *Ochlerotatus*, *Culex*, *Mansonia*, *Psorophora*, and *Culiseta*) are of major importance to human health. Culicine mosquitoes are primary vectors of a number of viruses and filariae and pose a serious problem as pest insects in many parts of the world.

Several species formerly recognized as members of the genus *Aedes*, the largest of the Culicine genera, have been undergoing a major reorganization. In 2000, the genus was divided into two genera, *Aedes* and *Ochlerotatus*, on the basis of consistent primary char-



Figure 38.9. *Aedes aegypti*, the yellow fever mosquito, in a typical feeding position.

acters of the female and male genitalia.^{20, 21} These changes have been generally accepted. A more dramatic renaming was suggested where common mosquitoes such as *Aedes aegypti* would be called *Stegomyia aegypti* and *Aedes albopictus* renamed *Stegomyia albopicta*. These changes are being hotly debated.

Mosquitoes of the genera *Aedes* and *Ochlerotatus* remain in the “tribe” Aedini, and are found in all habitats, ranging from the tropics to the Arctic. The typical aedine mosquito (Fig. 38.9) is robust, a strong flier, and usually a vicious biter. Its eggs are laid singly, without floats, on or near the surface of water or in areas likely to be flooded periodically. Unlike the eggs of *Anopheles* or *Culex* mosquitoes, which usually hatch within a few days of deposition, aedine eggs have the capacity for an extended period of dormancy. This dormancy allows the eggs to survive the winter or to delay hatching until conditions are ideal for development. Aedine mosquitoes occupy salt marsh habitats, flood plains, tree holes, irrigated pasturelands, and human-made containers.

Aedine larvae are nourished by food filtered from water. They develop and feed while suspended from the surface of water by a breathing tube. Larvae develop by progressing through four stages over a period of 6–10 days, or longer at lower temperatures. Aedine pupae are typical of those of most mosquitoes; this stage usually lasts less than three days. Adults usually emerge from breeding sites synchronously, followed by mass migrations of females in search of blood.

Aedine species may develop overwhelming populations in salt marshes, tundras, pastures, and floodwater, and they have a severe impact on wildlife, livestock, and humans. If left uncontrolled, the salt-marsh mosquitoes of the East Coast of the United States, *Ochlerotatus sollicitans* and *Oc. taeniorhynchus*,

could deny vast areas of seashore to development and tourism. The floodwater mosquito, *Ae. vexans*, develops after spring rain and flooding; the Arctic species begin hatching with the first melting snows. Populations of Arctic *Aedes* become so great at times that humans and larger mammals do not venture into the tundra area. In some Arctic species, the first egg batch is produced without need of a blood meal, a physiological adaptation termed autogeny.

Aedines, breeding in tree holes in the populated areas of temperate zones (e.g., *Oc. triseriatus*), seldom produce large populations, but can become local pests and important vectors of various viral infections. In tropical regions, populations of aedine mosquitoes are usually much smaller than in the Arctic. *Aedes aegypti* (Fig. 38.9), the yellow fever mosquito, occurs alongside humans throughout the tropics and subtropics. *Ae. aegypti* usually breeds in human-made containers such as discarded auto tires, flowerpots, blocked gutters, water jugs, rain barrels, cemetery urns, and tin cans. The mosquito lays eggs above the waterline in these containers, and the eggs remain dormant there, often as long as six months, until the container becomes filled with water. Because this mosquito is closely associated with humans and is almost exclusively anthropophilic, it has most of the characteristics of a good vector. *Ae. aegypti* is the primary vector of yellow fever and dengue in urban environments throughout the world. In the South Pacific, container-breeding members of the *Ae. scutellaris* complex are vectors of certain viruses and *W. bancrofti*. The introduction of *Ae. albopictus* and *Ae. (Ochlerotatus) japonicus* into the United States has added two species with the potential for transmitting dengue. Both of these introduced species are proving to be serious biting pests, particularly in urban and suburban areas of the Eastern and Southeastern United States. *Ae. albopictus* appears to be out-competing *Ae. aegypti* in many areas.

The genus *Culex* is the second largest group in the subfamily, best represented by *Cx. pipiens pipiens*, the northern house mosquito found in temperate areas, and *Cx. pipiens quinquefasciatus* (formerly known as *Cx. fatigans*), the southern house mosquito found throughout the sub-tropics and tropics.

Culex mosquitoes deposit their eggs in rafts, which usually contain 50–200 eggs cemented together. The eggs float perpendicular to the water surface and hatch within 2–3 days. The four larval stages develop and feed on nutrients in the water, much like aedine mosquitoes. The siphons of larval *Culex* mosquitoes are usually longer and more slender than those of Aedines. The larval period lasts less than two weeks and the pupal stage less than two days. Adults usually feed at night. Many show a preference for avian blood, but most members also feed on humans or other mammals. *Cx. p. quinquefasciatus* is the major vector of *W. bancrofti* throughout the tropics. The species is particularly well-adapted to development in polluted waters, breeding in or near population centers and readily biting humans.

The genus *Mansonia* includes a number of species important as vectors of Brugian filariasis. This genus differs in its development from most other mosquitoes in that its larvae and pupae affix themselves below the surface of water to the stems and roots of aquatic plants and derive oxygen from these plants. Mosquitoes of the genus *Psorophora* can be important biting pests. *Culiseta* includes several species involved in the transmission of arboviruses to humans.

Pathogenesis of the Mosquito Bite

The mouthparts of the adult female mosquito are adapted for piercing flesh and sucking the blood needed by the female for the production of eggs. During the act of feeding, the female

repeatedly injects saliva, which produces the reaction that follows the bite.²²

Although the mechanical damage induced by the feeding mosquito can cause pain and irritation, the immediate and delayed immune reactions are of greater concern. Individuals with no previous exposure to mosquitoes show neither immediate nor delayed reactions. After sensitization, a bite is followed by a small, flat wheal surrounded by a red flare, which appears within a few minutes, lasts about 1 hour and is mediated by antibodies. The delayed reaction consists of itching, swelling, and reddening of the wound region. It may persist for days. Eventually loss of the delayed reaction and desensitization can develop after repeated exposures. Desensitization to one species does not necessarily extend to other members of the same genus and usually does not include protection against the bites of mosquitoes of other genera. The intense itching, primarily associated with the delayed reaction, encourages scratching and secondary infection of the wound site. Local anesthetics are useful for treating reactions to mosquito bites.²³⁻²⁵

Mosquito-Borne Viral Diseases—The New Plagues of the 21st Century

Viral diseases transmitted to and between humans have evolved with humanity from its existence. Yellow fever and its primary vector, *Aedes aegypti*, established itself throughout the tropics with the earliest voyages of exploration and colonization. In the United States and Europe, the rapid spread of several previously restricted viral diseases along with the invasion of a least two efficient vector species have occurred over the last 30 years.

Aedes albopictus, the Asian tiger mosquito, is a common species in Japan and Korea. Mosquito eggs, carried in used automobile

tires, first invaded Houston, Texas where it was detected in 1985. From there it has spread throughout the continental United States and into Hawaii. By 1990, *Ae. albopictus* was established in Italy, by 1999 in France and by 2007 in Germany. *Ae. (Ochlerotatus) japonicus*, the Asian rock-pool mosquito, is also native to Japan and Korea. This invasive species was first detected in the northeastern United States in the late 1990's and has rapidly spread to most states east of the Mississippi River, plus Oregon, Washington and Hawaii and eastern Canada to the shores of Hudson's Bay. It is also established in Germany. Both species have become serious biting pests in urban and suburban backyards in the eastern United States.

Yellow fever, caused by a flavivirus, has historically been one of the most serious and widespread of the arboviral infections. The virus causes a severe hemorrhagic disease, characterized by high fever, jaundice, and prostration. Case fatality during epidemics may exceed 10%. The yellow fever virus naturally infects monkeys and is maintained in a monkey-to-monkey sylvatic cycle by forest-dwelling mosquitoes. When the sylvatic cycle is disturbed (e.g., by wood cutters), humans can be bitten by one of the monkey-feeding vectors. When these individuals return to their villages and become viremic, the ubiquitous *Ae. aegypti* initiates the urban cycle of transmission from person to person. An effective vaccine for yellow fever is available and is usually required for travelers to endemic areas.

Dengue, another flavivirus, is an acute, usually non-fatal viral disease characterized by high fever, severe headache, backache, and arthralgia. It is commonly known as "break-bone fever." A hemorrhagic form of dengue is frequently fatal. *Ae. aegypti* is the usual vector of both the typical and the hemorrhagic forms of dengue, although other aedine mosqui-

toes, particularly *Ae. albopictus* may transmit the organism. In 2015, Brazil recorded over 1.5 million cases of dengue with nearly 500 deaths. There is no verified animal reservoir for dengue; several vaccine candidates, which are effective against all serotypes of the virus, are being evaluated in clinical and field trials.^{26, 27}

In the United States, the mosquito-borne viral encephalitides include St. Louis encephalitis, eastern equine encephalitis and western equine encephalitis. They are viral diseases of wild birds transmitted by mosquitoes. Under certain conditions, normally ornithophilic (bird-feeding) mosquito species that had previously fed on viremic birds feed on humans or other mammals. Members of the *Culex pipiens* complex in urban areas may transmit St. Louis encephalitis, by *Cx. tarsalis* in rural areas in the western states, and by *Cx. nigripalpus* in Florida. *Cx. tarsalis* is the main vector of western equine encephalitis in the West, and *Culiseta melanura* is one of the major vectors of eastern equine encephalitis in the East.

Japanese encephalitis is transmitted by *Cx. tritaeniorhynchus*, *Ae. togoi* and *Oc. japonicus*. In Australia and New Guinea, Murray Valley encephalitis is transmitted by various *Culex* spp. Rift Valley fever, an East African disease usually associated with wild animals and livestock, caused a serious epidemic in Egypt in 1977–1978, infecting millions. The viral agent of Rift Valley fever has been assigned to the sand fly fever group of viruses and is probably transmitted in Egypt by *Cx. pipiens* and by other *Culex* and *Aedes* mosquitoes throughout the rest of Africa.

California group viruses, including La Crosse virus, rarely cause epidemics. The tree-hole breeding species *Oc. triseriatus* in the Midwestern United States transmits the California group viruses.

West Nile virus is a member of the flavivirus group responsible for regular epidemics in human populations in Europe and Africa. It can also cause significant epizootics in birds. The 1999 outbreak of human encephalitis in New York associated with the West Nile virus was the first isolation of this agent in the New World and was concurrent with extensive mortality in crows and other corvids. The vector that transmitted West Nile virus to people in the New York City environs was probably *Cx. pipiens*. This is the usual vector for bird-to-bird transmission. West Nile virus has remained endemic in the U.S., and outbreaks in 2003–2004 were the largest on record, infecting an estimated 2 million people and killing countless wild birds. It is hypothesized that dry, hot spells of weather of more than two weeks favor such outbreaks in humans.

West Nile virus vaccines have been developed for horses and are commercially available in the United States. A West Nile virus vaccine protective for humans is undergoing final clinical evaluation. It has yet to be determined if a human vaccine will ever be mass-produced for general use.

Two serious arboviruses, both of African origin, invaded the Americas in the second decade of the 21st Century. Chikungunya, an alphavirus, had already been seen in Ravenna, Italy in 2007, but appeared in several islands of the Caribbean in 2013. Thereafter, hundreds of cases were imported into the United States. Efficient vectors like *Ae. aegypti* and *Ae. albopictus* are present in the United States. In 2014, the first cases of transmission in the contiguous United States were reported in Florida.²⁸ In 2013, Zika virus, another of the flavivirus group was reported in French Polynesia and in 2015 appeared in Mexico and Brazil. This virus spread rapidly through the Americas and although symptoms are normally mild, many

cases of microcephaly developed in newborn babies, Guillain-Barré syndrome and acute disseminated encephalomyelitis have been linked to these infections. Again, the primary vectors are the peridomestic mosquitoes, *Ae. aegypti* and *Ae. albopictus*.

Control

The most effective method of mosquito control is reduction at the source (i.e., the elimination or modification of the aquatic sites at which the mosquitoes breed). Control may take the form of draining of impoundments, controlling the level of large bodies of water (via dams), the clearing or filling of ditches, and the elimination of human-made containers. Methodology must be tailored to the specific breeding requirements of the species. The general use of chemical insecticides has obvious potential for deleterious side effects. Given the serious nature of many of the mosquito-borne diseases, insecticide use may be required where reduction at the source is inadequate. Larvicides can be applied to breeding sites. Under extreme conditions, pesticides can be directed against adult mosquitoes.

The most common and effective method of malaria control employs insecticides applied to the walls of houses. Anopheline malaria vectors tend to rest on walls after feeding; they then come in contact with the residual insecticide and die. Consequently, insecticides applied to the insides of walls affect only those mosquitoes that have fed on humans and are potentially infected. This scheme does little to reduce mosquito populations and usually has little environmental impact; it does, however, reduce the incidence of malaria by interrupting transmission of the disease. DDT was effectively used in house spraying programs for several decades. In addition to toxicity to resting mosquitoes, this insecticide produced a repellent effect that discouraged mosquitoes

from entering treated houses. Bed nets, with or without insecticide impregnation, can provide significant protection from feeding mosquitoes.

Removing or destroying their breeding sites most effectively controls *Ae. aegypti*, *Ae. albopictus* and *Ae. Oc. japonicus*, the periurban vectors of dengue, chikungunya and Zika virus. Disposal of used automobile tires and tin cans, clearing gutters of standing water, covering rain barrels, and generally denying water containers to mosquito breeding is an important first step.

Control of *Aedes aegypti*, the primary vector of Zika virus, has taken a significant leap forward with the demonstration that the release of genetically modified male mosquitos can significantly reduce populations of this species. Field trials of this strategy in Panama, Brazil, and the Cayman Islands have been particularly promising with vector population reductions of over 90%.²⁹ The FDA has approved a field trial to be conducted in a suburb of Key West, Florida. In its approval the FDA noted that the program presents “no significant environmental impact”. If successful, the use of genetically modified sterile male mosquitos could provide an environmentally friendly method for controlling this important vector without the use of insecticides. However, a coalition of environmental public interest groups has mounted a campaign to block the field trials.

Recent studies have shown that oral ivermectin given to humans and domestic animals will kill anopheline mosquitoes, notably the major African vector *An. gambiae*, and could have a major effect on vector populations and malaria transmission. Systemic ivermectin appears to cause mortality in female *Ae. aegypti* and *Ae. albopictus*, but does not cause mortality in *Culex* mosquitoes.³⁰

A number of effective mosquito repellents are available as sprays or lotions. When applied properly, they can reduce the annoyance caused by the insects. The most effective repellents usually contain DEET.

Tabanidae: Horse and Deer Flies

The Tabanidae are a large family of blood-sucking dipterans with a cosmopolitan distribution. They are robust flies, ranging in size from 7–30 mm in length, and are locally referred to as horse flies, deer flies, or greenheads. Tabanids are strong fliers, capable of inflicting painful bites, and in some areas of the world are considered serious pests of humans and animals. Flies of the genus *Chrysops* (\KRIS-ops\, \kris-,äps\) act as vectors of the filarial eye worm *Loa loa* in Africa and may be involved in the mechanical transmission of anthrax, tularemia, and *Trypanosoma evansi*.

Historical Information

Tabanids were implicated in the transmission of anthrax as early as 1874, and of *T. evansi* in 1913. The role of tabanids as intermediate hosts and vectors of *L. loa* was verified in 1914 by Robert Leiper.³¹

Life Cycle

Tabanids usually lay eggs on vegetation near moist areas. Their larvae develop in water or wet earth and pass through four to nine stages. In some species the larvae remain dormant during the winter. Pupation occurs in dry earth, and the quiescent pupal stage may last 2–3 weeks. Adult females feed on blood and the males on plant juices.

Pathogenesis

Tabanid mouthparts are short and blade-like. During the act of biting, the insect inflicts a deep, painful wound, causing blood to flow. The fly then ingests blood from the freshly formed pool. Individuals can become sensitized to tabanid bites and suffer severe allergic reactions after attack.

Tabanids act as efficient mechanical vectors of several pathogens. They are easily disturbed during feeding. They fly to another host and begin the process anew. Consequently, the fly's mouthparts can readily transfer organisms to the next host after contamination on the first. Bacteria causing anthrax and tularemia, the protozoan *T. evansi*, and the retrovirus agents of bovine leukemia and equine infectious anemia may be transmitted by the tabanid flies, which act as mechanical vectors.



Figure 38.11. *Glossina* spp. tsetse fly feeding on blood. Courtesy of J. Gingrich.

L. loa is transmitted by African tabanids of the genus *Chrysops*, which include *C. silacea* and *C. dimidiata*. Microfilariae of the worm, ingested by female flies with the blood meal, develop in the flight muscles. When they reach maturity, they migrate to the mouthparts and are deposited on the skin of a new host when the fly feeds again. Infectious larvae burrow into the skin of the host after the fly has abandoned the bite wound.

Control

Tabanids are difficult to control because of their diverse breeding sites. Larvae are sensitive to DDT and other insecticides, but these compounds are seldom used. Sensitive individuals should consider using repellents to avoid bites. Mosquito repellents containing DEET are usually effective. Although DEET is the standard for mosquito repellents there are some people that are not able to tolerate DEET and alternatives such as picaridin are available.

Muscidae: The House Fly and its Relatives

The muscoid flies include insects that are important as blood-sucking pests, vectors of diseases, and mechanical vectors of a variety of pathogenic organisms.^{32, 33} Some better-known members of this family are the housefly *Musca domestica* and the stable fly *Stomoxys calcitrans*.

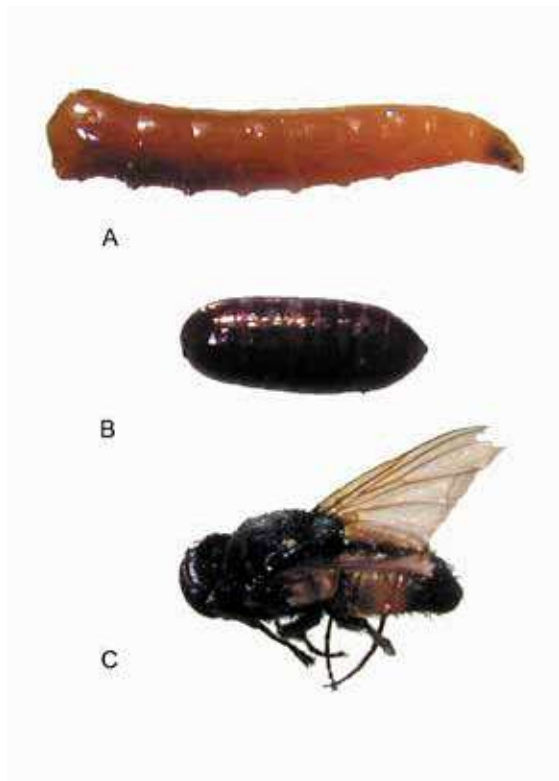


Figure 38.10. *Musca domestica*, the housefly. A. Larva. B. Pupa. C. Adult. The larvae of flies are referred to as maggots.



Figure 38.12. *Sarcophaga* larvae *in situ* (Courtesy of Y. Mumcoglu. In Mumcoglu Y, Ruffli Th: Dermatologische Entomologie. Perimed Fachbuch, Erlangen, 1982).

Life Cycle

Musca domestica (Fig. 38.10), the ubiquitous housefly, lays her eggs on any matter that will serve as food for the developing maggots. Animal or human feces, garbage, decaying plant material, and sewage all provide suitable substrates. A single fly lays more than 1,000 eggs during her life span. The development from eggs to adults requires less than 10 days at summer temperatures. As a result of this reproductive potential, summer fly populations can be enormous. These flies can carry viruses, bacteria, protozoa, and the eggs of parasitic worms, and are a serious public health problem.^{35, 36} The presence of large fly populations is a clear indicator that sanitation is needed. While a person may have an otherwise clean home, if an animal dies or there is another localized source, this can be indicated by an increased number of flies.

Stable Flies

Most muscoids are fairly large, robust dipterans. They develop from eggs to maggots (larvae), non-motile pupae, and adults by complete metamorphosis. Only the tsetse flies differ, in that their larvae develop singly within the female and are deposited fully developed and ready for pupation.

Historical Information

One of the plagues of Egypt described in the Old Testament consisted of swarms of flies. It appears that these insects have troubled humans throughout history. In 1895, David Bruce demonstrated the role of the tsetse fly as the vector of African trypanosomiasis while, in 1898, M.A. Veeder demonstrated the importance of houseflies as disseminators of various pathogens.^{33, 34}

Stomoxys calcitrans is a serious biting pest usually associated with domestic animal husbandry. The fly lays her eggs in moist, decaying vegetable material (e.g., hay, alfalfa, straw, and manure). In suburban communities, moist piles of grass clippings and weeds provide ideal sites for larval development. The egg-to-adult period during the summer lasts about 4 weeks, and a female may lay as many as 400 eggs during her life span.

Although superficially similar in appearance to houseflies, stable flies have a prominent proboscis, which both sexes use effectively for sucking blood. The bite of the stable fly is initially painful but usually causes little delayed reaction. Sensitized individuals develop allergic responses to repeated bites. *Stomoxys* serves as a mechanical vector for anthrax and some trypanosomes of animals.³⁴



Figure 38.13. Myiasis: note the opening (black spot) in the skin which permits the maggot, burrowing in the tissue below, to breathe.

Glossinidae: The Tsetse Fly

Tsetse Flies

Tsetse flies of the genus *Glossina* occur in Sub-Saharan Africa, where they are the intermediate hosts and vectors of a number of trypanosomes infecting humans and animals (Fig. 38.11). Tsetse flies differ markedly from muscoid flies, and indeed from most insects, in that they produce only one egg at a time. This single egg is retained within the “uterus” of the female, where it hatches. The larva develops in three stages “*in utero*” while feeding on “milk” produced by accessory glands of the female. Eventually, a fully mature larva is deposited in a shady location, and it pupates immediately. The pupal stage can last up to 30 days and the resulting adult remains inactive for 1–2 days after emerging to seek its first blood meal. Both male and female tsetse flies are exclusively hematophagous, and both sexes are capable of transmitting trypanosomes.^{37, 38}

A female tsetse produces 10–15 larvae during her life span. Tsetse populations are relatively small and dispersed. *Glossina* hunt by sight and follow animals, humans, or even vehicles for long distances. They feed during the day,

usually along paths or riverbanks. *G. palpalis* and *G. tachinoides* are the main vectors of *Trypanosoma brucei gambiense*; *G. morsitans*, *G. swynnertoni*, and *G. pallidipes* are the primary vectors of *T. b. rhodesiense*.

Calliphoridae, Cuterebridae, and Sarcophagidae: Myiasis-Causing Flies

Not all dipterans inflict damage by the bite of adult flies seeking blood. The larvae of several families are pathogenic during their development within the tissues of the infested host. This infestation with larvae, or maggots, is known as myiasis.³⁹ Certain species of flies are obligate parasites and require living tissue for development. Other species develop facultatively in either living or dead tissues. A third group can cause accidental myiasis when their eggs, deposited on foodstuffs, are ingested. Cheese-skippers of the family Piophilidae, rat-tailed larvae of the Syrphidae, soldier fly larvae of the Stratiomyidae, and several species of the Muscidae cause gastrointestinal myiasis. Symptoms are proportional to the number of larvae developing and include nausea and vomiting. Diagnosis



Figure 38.14. Myiasis. Maggots of *Cordylobia anthropophaga* in the flesh of an infant. Note the raised areas and openings for the larvae to breath.

requires the finding of living or dead maggots in the vomitus, aspirates of gastrointestinal contents, or stool specimens.

Species of flies that normally favor decaying flesh for larval development occasionally deposit eggs or larvae on wounds or ulcers (Fig. 38.12).

Maggot therapy is the use of the larvae of certain fly species for selectively debriding non-healing necrotic skin and soft tissue wounds.⁴⁰ In 2004, the FDA began regulating the medicinal use of maggots.^{41, 42} In Europe, thousands of maggot treatments are applied annually.

The flesh flies of the family Sarcophagidae contain several members of the genera *Wohlfahrtia* and *Sarcophaga*, which cause myiasis. Female flies in this family do not lay eggs, but deposit freshly hatched first-stage larvae directly in wounds, ulcers, or even unbroken skin. These feeding larvae may cause considerable tissue damage.

Flies of the family Cuterebridae are obligate parasites, usually of wild and domestic animals. Human myiasis due to infestation with maggots of *Cuterebra*, normally associated with rodents, is not uncommon in the United States. This condition usually presents as individual larvae developing on various parts of the body (Fig. 38.13). *Dermatobia hominis*, the human botfly, parasitizes a number of mammals and is a serious pest of cattle in Central and South America. Flies of this species cause infestation in a unique manner. Female *Dermatobia* flies capture various blood-sucking arthropods (usually mosquitoes or other flies), lay their eggs on the abdomens of their prey, and release these insects. When the fly or the mosquito carrying the eggs alights on a warm-blooded host, the eggs hatch, immediately liberating larvae onto the skin of the host. These maggots pen-

etrate the skin and develop in the subcutaneous tissue, maintaining contact with the surface through a small opening in the center of an abscess-like swelling (Fig. 38.13). When the larvae complete their development after 6–12 weeks, they emerge, fall to the ground, and pupate. During the phase within the tissues, the maggots can cause intermittent pain and secrete a foul-smelling material from the opening in the skin.

For human infestations, each maggot should be removed surgically. Particular care must be taken not to damage it during the procedure because the patient has usually become sensitized to the antigens of the maggot. The maggots can also be removed by coating their external spiracles with petroleum jelly, which blocks access to oxygen. They are thus forced to crawl to the surface. These may then have to be removed surgically, under local anesthesia; and the wound left open and cleaned daily until healed.

Several species of the family Calliphoridae are obligate parasites, whereas others cause only accidental myiasis. *Cordylobia anthropophaga*, the tumbu fly, is an uncommon larval parasite of humans but can frequently infect animals, especially rats, in Africa. These flies lay eggs on soil contaminated with



Figure 38.15. Body lice after feeding, resting on cloth.



Figure 38.16. Crab louse. Photo David Scharf.

urine or feces, or on similarly soiled bedding or clothing that is set out to dry. The emerging larvae attach themselves to any host with whom they come in contact and penetrate the skin. After penetration, larvae cause individual tender abscess-like swellings from which serous fluid exudes, particularly when pressure is applied to the lesion (Fig. 38.14). Treatment consists of covering the wound with petroleum jelly to force the maggot to the surface in search of oxygen. The maggot can then be gently squeezed out. Surgical excision is necessary for some infestations.

Another African species, the Congo floor maggot, *Auchmeromyia luteola*, feeds preferentially on humans. The fly lays eggs on the floor of huts. The maggots come out of the soil at night to feed on the blood of the inhabitants of the hut who sleep on the floor. The larvae lacerate the victim and suck blood but do not penetrate tissues, returning to the soil after taking their blood meal.

Two species of *Cochliomyia*, the New World screwworm, occasionally cause myiasis in humans in North and South America, although these flies are primarily parasites of animals. Adult females lay their eggs around the edges of wounds, and the larvae invade the wounds and macerate the traumatized tissues.

Large numbers of maggots can infest a single wound. Because infestations of the nose can be fatal, the maggots should be removed surgically as soon as they are detected. Screw-worms continue to be a problem for veterinarian practitioners and have been reintroduced into certain states in the U.S. (e.g., Florida).

Flies of the genus *Chrysomyia*, the Old World screwworm, are important causes of human and animal myiasis throughout Asia and Africa. *Chrysomyia* larvae penetrate wounds or mucous membranes, primarily affecting areas around the eyes, ears, mouth, and nose.

Green bottle flies (*Lucilia* spp.) and blue bottle flies (*Calliphora* spp.) sometimes infest wounds of humans in Asia, Africa, and the Americas. The larvae of these species prefer dead tissue; in the past, these maggots, reared free of pathogens, were used therapeutically for cleansing septic wounds.⁴³⁻⁴⁵ A number of flies whose larvae are primarily parasites of domestic animals occasionally infest humans. Larvae of the sheep botfly (*Oestrus ovis*) may invade nasal cavities of shepherds and cause severe frontal headaches.⁴⁶ Such larvae do not complete their development because humans are aberrant hosts, so the larvae usually exit spontaneously before maturation.

Cattle warbles of the genus *Hypoderma* occasionally infest humans, causing a condition similar to creeping eruption. Larvae penetrate exposed skin and wander aimlessly, causing severe itching, pain, and sleeplessness. Surgical removal of the larvae from the ends of their burrows is recommended.

Larvae of various flies, particularly of the genera *Calliphora*, *Phaenicia*, and *Cochliomyia*, infest a cadaver in a predictable succession. The science of forensic entomology has developed the use of flies and, to a lesser extent, beetle larvae to determine the manner, time, and place of death; it uses entomologic

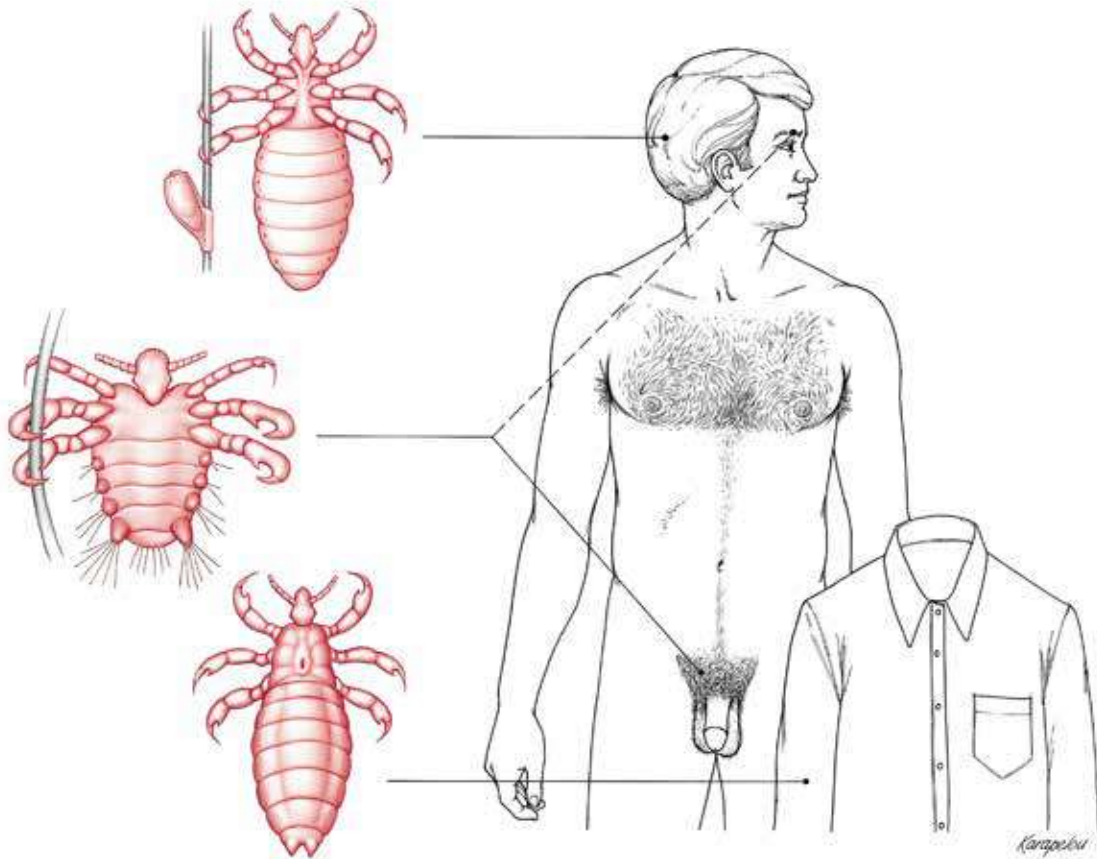


Figure 38.17. Preferred feeding and resting sites of the three species of louse affecting humans. *Pediculus humanus capitis*, the head louse, resides, feeds, and reproduces on the hairs of the head. Eggs are laid individually on hair shafts. *Phthirus pubis*, the crab louse, prefers hair of the pubic regions but is occasionally found on the eyebrows, eyelashes, beard, or moustache. Eggs are attached to the individual hairs. *Pediculus humanus humanus*, the body louse, is usually found on clothing, moving to the body of the human host only to feed. Eggs are laid in masses in the seams of the clothing of the host.

information to support pathologic findings in legal proceedings.^{32-34, 47-49}

Anoplura: Sucking Lice

Three species of lice infest humans as obligate, blood-feeding ectoparasites. Only one of them, the body louse, is important in human medicine as the vector of the rickettsiae of epidemic typhus and trench fever and the spirochetes of relapsing fever. Louse infestation is known as pediculosis.⁵⁰

The body louse, *Pediculus humanus humanus* (Fig. 38.15), and its close relative, the head louse, *P. humanus capitis*, are wingless, elongate, dorsoventrally flattened insects, 2.5–4.0 mm long. They have three pairs of legs of about equal length. Their mouthparts are adapted for piercing flesh and sucking blood. The crab louse, *Phthirus pubis* (Fig. 38.16), is shorter (0.8–1.2 mm) and, as its common name implies, resembles a crab. Crab lice have somewhat reduced front legs, with the second and third leg pairs stout and strongly clawed. All lice undergo development characterized by incomplete metamorphosis.

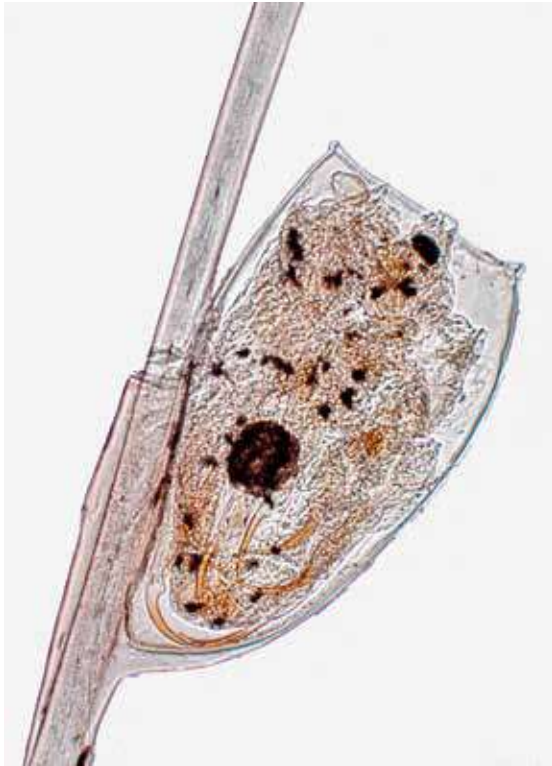


Figure 38.18. Nit of a louse attached to a shaft of hair.

Historical Information

The association between humans and lice is an ancient one and probably represents an evolutionary relationship begun by lice and ancestral hominids. Closely related species of lice infest gorillas and monkeys. Humans have certainly been aware of the discomforts of louse infestation from the earliest times, and the condition has been recorded by poets and artists, as well as by early writers on science and medicine. The recognition of body

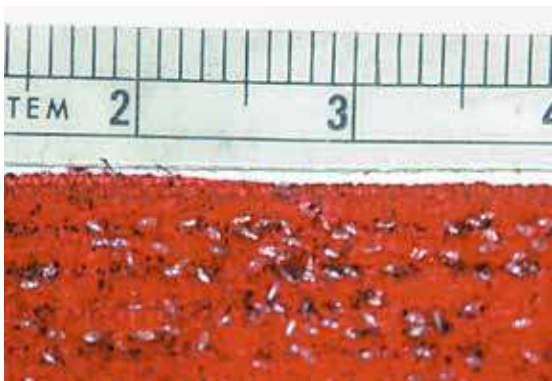


Figure 38.19. Body louse eggs on fabric.



Figure 38.20. Louse bites.

lice as disease vectors is more recent. Transmission of typhus and relapsing fever by lice was not demonstrated until the early 1900s.

Lice have been considered variously as unwelcome pests or a sign of unclean habits. They have often been accepted as one of life's unavoidable afflictions. As vectors of diseases, body lice have, on numerous occasions determined the outcome of human history. Zinsser, in 1935, and Busvine, in 1976, chronicled empires and even entire civilizations that were profoundly changed by epidemics of louse-borne typhus.^{51, 52}

Life Cycles

The preferred feeding and resting sites of the human lice are depicted in Figure 38.17. The crab louse, *P. pubis*, sometimes referred to as *papillion d'amour*, usually inhabits the hairs of the pubic and perianal regions of the body but can also be found on axillary hair or on moustaches, beards, eyebrows, or eyelashes. Adult crab lice are sedentary, often clutching the same hairs for days while feeding for hours at a time. Lice of all stages and both

sexes feed solely on blood. They must obtain daily blood meals to survive.

The female lays individual eggs or nits (approximately 0.6 mm in length) and attaches them to hairs of the host (Fig. 38.18). They embryonate and hatch over 6–8 days. The louse has three nymphal (pre-adult) stages, lasting 15–17 days, before the final molt to the adult stage. Nymphs are tiny, sexually immature versions of the adults. Adult crab lice live less than one month, and the females usually lay fewer than 50 eggs during their lifetime. The entire life cycle (i.e., egg-to-egg interval) lasts 22–27 days.^{40, 53, 54}

Crab lice are most frequently transmitted from one person to another by sexual contact. While it may be possible that general physical contact or contact with a variety of contaminated objects such as clothing, or bedding might result in rare cases of infestation it would be extremely unlikely to contract pubic lice from sitting on a toilet seat. This is because lice are unable to live for very long away from the warmth of a human host and do not have appendages that allow them to successfully navigate smooth surfaces.⁵⁵

The head louse, *P. humanus capitis*, inhabits the hairs of the head, particularly behind the ears and around the occiput. Heavy infestations may force head lice to establish themselves on other hairy parts of the body. Like the crab lice, the head lice are relatively sedentary, feeding for hours at a time while clutching firmly to hair; like the crab lice, they seldom leave the hairy regions voluntarily. The eggs are attached to hair shafts and hatch within approximately one week; the three nymphal stages are completed within less than 14 days. The egg-to-egg cycle lasts about three weeks. A head louse lays 50–150

eggs during her lifetime. Head lice are disseminated by head to head contact between individuals. These pests do not tend to move to inanimate objects, as they cannot survive away from a host for very long. Head lice are not typically spread by sharing of hats, scarves, or by the common storage of garments.⁵⁶

Head lice have clasping legs that have been modified through evolution to hold onto human hair shafts. Although head lice have been shown to be capable of transmitting rickettsiae and spirochetes in the laboratory, they are usually not involved in the transmission of these organisms under natural conditions.

The life cycle of the body louse, *P. humanus humanus*, differs significantly from those of the other two in that body lice spend much of their lives on the clothing of infested individuals. Body lice (commonly referred to as “cooties”) are usually found on clothing wherever it comes into close contact with the body. Although body lice in all stages of their development must move to the body for regular blood meals, they return to the clothing after feeding. The lice lay eggs along the seams of garments attached to cloth fibers and sometimes attach the eggs to some of the coarser body hairs (Fig. 38.19). Eggs kept near the body hatch within 5–7 days. Nymphs require about 18 days to mature, and the adult lice live for about a month. A body louse lays more than 300 eggs during her lifetime.

Body lice are readily transmitted between individuals by physical contact, exchanges of clothing, or the common storage of infested garments. They are the only vectors of louse-borne relapsing fever, trench fever, and epidemic typhus.

Pathogenesis

Lice inject salivary fluids into the wound during ingestion of blood. These secretions induce varying degrees of sensitization in the human host.

Clinical Disease

The usual characteristic of infestation by all types of lice is intense itching. Constant scratching can lead to secondary bacterial infection of the wound. Crab lice produce characteristic “blue spots”, which are often seen around the eyes of individuals with infested eyelashes. The bites of head lice result in inflammatory papules and impetiginous lesions often associated with lymphadenopathy (Fig. 39.20). Heavy infestations of head lice can cause a condition in which hair, eggs, louse feces, and exudates of bite wounds form a cap-like mass teeming with lice. There may be secondary fungal infection within the mass.⁵⁷ Children infested with head lice often appear restless.

Bites by body lice cause pinpoint macules, excoriations, and pigmentation of the skin. “Vagabond’s disease” is an extreme condition caused by a combination of persistent heavy infestation and poor personal hygiene. Affected individuals show a generalized bronze pigmentation and hardening of the skin.⁵⁸

Diagnosis

The diagnosis depends on identification of lice or eggs in the hair or in the seams of garments. In the latter, they may be difficult to find. The eggs must be identified by microscopy. For the detection of head lice it is critical to employ the wet combing approach as it has a much higher sensitivity for detecting active infestation.⁵⁹

Treatment

There are several formulations available as dusts, shampoos, lotions, and creams. Some may be obtained as over-the-counter preparations, and others require a prescription. All of the effective products contain low concentrations of insecticides such as benzene hexachloride, pyrethrum, or synthetic pyrethrum analogues (e.g., permethrin).⁶⁰

Head and crab lice can be treated similarly. Infested individuals should remove all clothing, apply the pediculicide, and put on clean clothing after treatment. The procedure should be repeated after 10 days to kill any newly hatched lice, as most treatments do not kill eggs. To prevent re-infestation, the clothing and bedding of infested individuals should be dry-cleaned or washed and dried by exposure to heat. Exposure of infested clothing to temperatures of 70 °C for 30 minutes kills lice and eggs. Combs and brushes should also be treated by heat to prevent re-infestation by head lice. Simply washing the head or affected areas with soap does not kill lice or destroy the nits.

Currently treatment with permethrin 5% cream applied to the entire body for 8–10 hours is recommended for treatment of body lice. Permethrin 1% is recommended for head lice. Benzene hexachloride (lindane, Kwell), although one of the most effective treatments for head lice, is now reserved for second line therapy due to concerns regarding its toxicity.⁶¹ Oral ivermectin as a systemic insecticide has been suggested as an alternative therapy.^{61–66} Insecticides should not be used on crab lice infesting eyebrows or lashes. Petroleum jelly should be applied thickly, and individual lice removed with forceps.

Because body lice inhabit and lay eggs on clothing, regularly changing underwear and

garments significantly reduces the infestation. Garments infested by lice should be treated as indicated above. Blankets, bedding, sleeping bags, and other items that might be contaminated should be similarly treated.

Various powdered formulations of pediculicides can be applied directly to clothed individuals. Several of these compounds have been used effectively for mass treatment of large groups of infested individuals to control epidemic typhus. Nit combs, hair combs with teeth spaced closely enough to scrape the louse eggs (nits) from the hair, can be effective if used thoroughly and repeatedly. All nits must be removed to prevent re-infestation.

Epidemiology

The three species of human lice can be considered cosmopolitan in distribution, with infestations recorded throughout tropical, temperate, and Arctic regions. The absence of lice in a population is a result of social or hygienic habits rather than of geographic or climatic factors.

The rates of infestations with crab lice are usually much lower than those for head or



Figure 38.21. *Pulex irritans*.



Figure 38.22. *Xenopsylla cheopis*, an important vector of plague.

body lice. Infestations with head lice can reach epidemic proportions, particularly among schoolchildren.^{67, 68}

Infestations with body lice are usually associated with poverty, crowded conditions, social upheavals such as wars, or natural disasters. Because body lice reside and deposit eggs on clothing, conditions that prevent changing and cleaning garments coupled with close contact and crowding foster the spread of these insects.

Louse-Borne Diseases

Body lice are the only vectors involved in infecting humans with *Rickettsia prowazekii*, which causes epidemic typhus, *Rochalimaea quintana*, the rickettsial agent of trench fever; and *Borrelia recurrentis*, the spirochete that causes louse-borne relapsing fever.⁶⁹

The rickettsiae multiply within the louse in the epithelial cells of the midgut, which ultimately rupture, releasing large numbers of these microorganisms. Human infections occur by rubbing infected louse feces into skin abrasions caused by the original louse bites. Scratching often extends these abrasions. Inhalation of fomites containing rickettsiae also causes human infection. Rickettsiae survive dehydration and remain infective for over two months at warm temperatures.

Humans are the usual reservoir for the rickettsiae of epidemic typhus. The organism can remain latent for years, occasionally giving rise to a mild recrudescent form of typhus termed Brill-Zinsser disease. Lice feeding on people with this form of typhus can become infected with the rickettsiae and transmit them to non-immune individuals, giving rise to the primary epidemic form of the disease. Studies have demonstrated a sylvan cycle for *R. prowazeki* in flying squirrels in the United States, but the importance of this rodent reservoir in the spread of typhus is yet to be determined.

Trench fever is a self-limiting disease caused by *Bartonella quintana*. Transmission to humans is similar to that of epidemic typhus. Individuals with trench fever can infect lice from the third day of illness and sometimes for months thereafter. The rickettsiae develop only within the cuticular margin of the louse gut (i.e., not intracellularly) and cause no disease in the insect. Infected feces and crushed lice are the usual sources of infection. The human is the only animal in which this rickettsia causes disease.

Louse-borne relapsing fever is caused by the spirochete *B. recurrentis*. The body louse is the only vector of *B. recurrentis*, although



Figure 38.23. *Ctenocephalides felis*. Photo D. Scharf.



Figure 38.24. *Tunga penetrans* in skin. Courtesy G. Zalar.

similar spirochetes cause tick-borne relapsing fevers. Lice are infected when feeding on infected individuals during febrile periods. The spirochetes invade the epithelium of the gut and ultimately the blood of the louse. Transmission can occur only when crushed lice are rubbed into a wound or are inhaled. Lice do not pass the spirochete by biting and do not excrete it in feces.

Siphonaptera: The Fleas

The Siphonaptera comprise a small order of insects. The adult fleas exist as ectoparasites on warm-blooded animals. The typical adult flea is a brown, laterally compressed, wingless insect with a tough skin, usually less than 3 mm long. Its third pair of legs is adapted for jumping, and it has mouthparts designed for blood sucking.

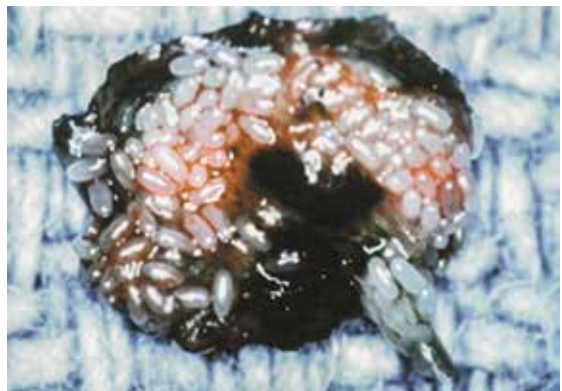


Figure 38.25. *Tunga penetrans* eggs. Photo G. Zalar.

Fleas undergo complete metamorphosis in their development, exhibiting markedly different larva, pupa, and adult stages. The larvae are delicate, motile, vermiform creatures; the pupae are encapsulated and quiescent.

Fleas transmit a number of diseases to humans. They are serious biting pests and vectors of a number of infectious agents, most notably the agent of bubonic plague, *Yersinia pestis*. Fleas usually feed quickly and to repletion often producing a cluster of bites at one site.

Historical Information

Humans have evolved with these “lair” parasites of domestic animals and fellow cave dwellers. Literature is replete with songs, poems, and stories extolling the virtues and vices of fleas and the miseries they cause. The importance of fleas as vectors was not recognized until the final years of the nineteenth century, when they were implicated in the transmission of plague. The historical impact of flea-borne bubonic plague, or Black Death, in the development of civilization, has been well-documented.^{51, 52}

Life Cycle

The life cycle of a typical flea is shown in Figure 38.3. Fleas are usually parasites of animals inhabiting nests, dens, or caves. The adult flea is an obligate parasite of its warm-blooded host, feeding only on blood. The flea scatters its eggs in and around the nest of its host. Larval fleas are active, yellowish-white creatures with biting mouthparts. They feed on flea dirt (flea feces) and each other. Under ideal conditions of temperature and humidity, eggs can embryonate and hatch in less than a week; larvae develop to adults in less than two weeks. After the flea has developed through three larval stages, it spins a cocoon and forms a quiescent pupa. The period of

pupation, during which the insect gradually develops its adult characteristics, may last from a week to a year depending on the species and the environmental conditions. The pupa, encased in its cocoon, can remain dormant for months. The quiescent adult, encased in the pupal cocoon is stimulated to emerge by detecting vibrations in the local environment, thus giving rise to a hungry adult flea.

Pathogenesis

The response to repeated flea bites is typical of reactions to most insect bites. Initial exposure produces little or no reaction, but after an individual is sensitized to the salivary antigens of the flea, first delayed reactions and then primary reactions develop.

Although many species of flea bite humans if the insects are sufficiently hungry, only a small number are consistent human pests. The combless fleas, so called because they lack prominent spines (ctenidia) on their heads, include several species that regularly feed on humans.

The human flea *Pulex irritans* (Fig. 38.21) is an ectoparasite of humans and animals, particularly swine. *P. irritans* is cosmopolitan in distribution and is the most common flea affecting humans. A closely related species, *P. simulans*, is restricted to the New World. Both species are capable of transmitting plague but are considered minor vectors of this disease.

The oriental rat flea, *Xenopsylla cheopis* (Fig. 38.22), as the vector of *Y. pestis*, has long been considered one of the great killers of humankind. It is an ectoparasite of rats, feeding on humans only when its customary host is unavailable. Classically, human bubonic plague is a consequence of an epizootic outbreak of plague in the rat population. As rats

die in massive numbers, infected fleas leave their dead hosts and seek fresh sources of blood. Under these circumstances, humans are readily attacked and infected.

Xenopsylla acts as an efficient vector because of its association with rats (its main reservoir) and its readiness to feed on humans. When a flea takes a blood meal from an infected rat, the plague organism rapidly multiplies within the flea's proventriculus, an organ of the intestinal tract lined with spiny projections. Within three days, the proventriculus is blocked by a gelatinous mass of partially digested blood and bacteria. When the flea feeds again, it is unable to engorge and is forced to regurgitate the blood and bacteria from the proventriculus into the host. Because the flea is unable to feed completely, it moves from host to host, repeatedly attempting to feed without attaining satisfaction, and transmitting the plague organism as it goes. The flea eventually dies of starvation, but not before its role as a vector of plague has been discharged.

The combed fleas also include species that affect humans. The dog and cat fleas, *Ctenocephalides canis* and *C. felis* (Fig. 38.23), are closely related, morphologically similar spe-

cies, with two sets of prominent combs on the head. Both species feed equally well on dogs and cats, and both bite humans if given the opportunity. Their larvae and pupae are usually found in the places where the animals rest. The fleas can prove particularly annoying when there are no pet hosts and humans are their only source of blood. Raccoons transfer dog or cat fleas into homes, often by building their nests in chimneys.

The northern rat flea, *Nosopsyllus fasciatus*, and the squirrel flea, *Diamanus montanus*, are common combed fleas of rodents in North America. They readily bite humans and may be involved in transmission of plague from wild rodents to rats or humans.

Tunga penetrans, known as the chigger flea or chigoe, is a serious pest in the tropical and subtropical regions of the Americas, Africa, and the Indian subcontinent.⁷⁰⁻⁷⁴ This flea originated in South America and was introduced into Africa during the late nineteenth century. Adult chigoes are less than 1 mm long. Both sexes feed regularly on blood. After insemination, the female flea attaches itself to the skin of the toes, soles of the feet, or the legs, and becomes enveloped by host tissue (Fig. 38.24). Thus protected, the female swells to the size of a pea, produces 150–200 eggs (Fig. 38.25), and dies still embedded in the tissue.^{75, 76} The infested tissue can become ulcerated and infected by bacteria, possibly including the clostridia, and cause tetanus or gas gangrene. Auto-amputation of toes is not uncommon. Wearing shoes is usually an effective means of preventing infection with *T. penetrans*. Treatment consists of removing the flea with a sterile instrument and treating the wound locally to prevent infection.

Clinical Disease

Intense irritation that leads to scratching and secondary bacterial infections is the main



Figure 38.26. Bed bug adult. *Cimex lectularius* 15x.

manifestation of flea bites. The major health problem caused by fleas is the transmission of infectious agents for which the fleas are vectors.

Diagnosis

The typical fleabite first appears as a single papule. With heavy flea infestations, papules may be grouped along the arms and legs, on the face and neck, or where clothing fits snugly. Precise incrimination of fleas requires capture of one of the offending insects. The species of the flea can be determined with the aid of a dissecting microscope and a key to identifying fleas that affect humans.

Treatment

Pruritus can be treated symptomatically. Secondary bacterial infection is medicated as appropriate.

Control

Flea control centers first on sanitation. Fleas can be controlled at the source of the infestation by various commercially available insecticides. Dusts should be applied to the fur and beds of dogs and cats. These dusts are particularly effective against the fleas that dwell in nests, whose larvae feed on particles. Space sprays can be effective against adult fleas. Pets can be treated for flea infestations topically or with systemic compounds that

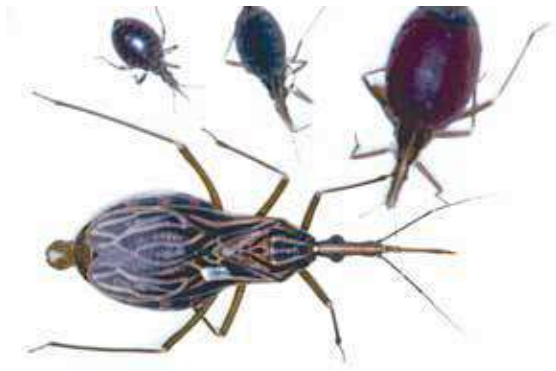


Figure 38.29. Kissing bug family of *Rhodnius prolixus*.

are lethal to the feeding insect. A number of topically applied repellents can protect individuals against fleabites for short periods.

Epidemiology of Flea-Borne Diseases

Fleas serve as primary vectors of *Yersinia pestis*, the agent of the plague, and *Rickettsia typhi*, which causes murine or endemic typhus. Fleas can serve as intermediate hosts of various cestodes and nematodes that infect mammals, including humans.

In 1898, Paul-Louis Simond performed an elegant experiment in Karachi (modern-day Pakistan) in the Hotel Reynolds where he determined that the rat flea was the vector for *Yersinia pestis*.⁷⁷

Yersinia pestis persists in nature in a so-called sylvan or campestral cycle in which wild rodents are constantly fed upon by various species of fleas. Foci of affected rodents occur in central Asia, South Africa, South America, and the Russian steppes. In western North America, plague is maintained in a ground squirrel reservoir with the squirrel flea, *Diamanus montanus*, as the vector. A number of other rodent species and flea vectors may also be involved. The United States reports 5–10 autochthonous cases of



Figures 38.27. and 38.28. Bed bug bites: note hemorrhagic bullae and paired bitemarks.

bubonic plague each year, usually among campers, hunters, and farmers in the western states. As long as plague exists as a disease of field rodents, human cases are rare. When the plague is transferred from wild rodents to peridomestic rats and becomes established in a rat-rat flea cycle, the potential for human infection increases markedly. The epidemiology of murine typhus has been clarified with the demonstration that *R. typhi* can be transmitted transovarially from an infected flea to her progeny.⁷⁸

Hemiptera: True Bugs

Pronunciation: \hi-'mip-tə-rə\

Hemiptera (\heh-MIP-tah-rah\) is an order with four suborders: Auchenorrhyncha, Coleorrhyncha, Heteroptera, and Stenorrhyncha. The two families of medical importance are Cimicidae and Reduviidae. Adults of most heteropterans are winged. The Cimicidae are not wingless but have wing buds rather than full wings. Bugs have mouthparts modified for piercing and sucking. Their long, slender, segmented beak is usually held along the ventral surface of the body when it is not in use. Most heteropterans are plant feeders, and some are predaceous on other insects; the ones that affect humans, the Reduviidae and Cimicidae are hematophagous. Heteroptera develop by incomplete metamorphosis.

Cimicidae: Bed Bugs

Three closely related species of bed bugs are blood-feeding ectoparasites of humans.⁷⁵ The common bed bug (*Cimex lectularius*) and the tropical bed bugs (*Cimex hemipterus*) are morphologically similar; they are oval, flattened, reddish-brown insects with mouth parts well-adapted for piercing flesh and sucking blood. Adult bed bugs (Fig. 38.26)

have non-functional reduced wings. They are approximately 5 mm by 3 mm. Their five nymphal stages are smaller, sexually immature copies of the adults.⁷⁶

Bed bugs are cosmopolitan in distribution. *C. lectularius* is widespread throughout temperate and tropical regions. The tropical bed bug, *C. hemipterus*, is restricted to tropical and sub-tropical climates.

Historical Information

Bed bugs evolved from ectoparasites of cave-dwelling mammals, probably bats, at the time when humans were also cave dwellers. Early Greek and Roman writers first recorded them as a problem in the Mediterranean region. In northern Europe they were identified much later (e.g., during the eleventh century in England).^{40, 52} Bed bugs have been suspected of transmitting a number of human diseases, but no direct evidence of involvement exists. Epidemiologic associations with hepatitis B virus transmission have not been verified experimentally.⁷⁹

Life Cycle

Bed bugs are found in a variety of human habitations, including homes, hotels, dormitories, prisons, barracks, and hospitals. They remain hidden in cracks and crevices in walls, floors, and furniture, usually appearing at night or in dim light to feed on a sleeping host. Humans are the preferred source of blood, but bed bugs feed on a variety of animals if humans are unavailable. They characteristically bite three or four times in succession over a period of a few minutes to engorge themselves.

Adult females lay 2–3 eggs per day for a total of 200–500 during a lifetime. The pearly

white eggs are 1 mm long. They are laid individually in crevices, behind loose wallpaper, in cracks in woodwork or furniture, or in mattresses. Hatching depends on temperature but usually occurs after 9–10 days of embryonation. There are five nymphal stages, each requiring a blood meal before molting to the next. The egg-to-egg period, depending on temperature, varies from 7–19 weeks, which allows the development of several generations of bugs within a year. Availability of a blood source also influences generation time.

Pathogenesis

Feeding bed bugs inject salivary fluids to sensitize the host. Primary exposure produces little or no reaction. After repeated exposures a severe delayed reaction may develop; continued exposure produces an additional primary reaction. Finally, after a course of regular exposures to bites, the host can become desensitized.

Clinical Disease

Reactions to bites can be mild or severe. Some individuals may never show a reaction even if repeatedly exposed and bitten. Large hemorrhagic bullae form on some sensitized individuals, whereas others develop erythema and local edema, and may experience severe, prolonged pruritus. Scratching can lead to secondary bacterial infections. Heavy bed bug infestations can interfere with sleep.

Diagnosis

The bite wounds are firm, closely spaced papules, appearing as variable numbers of lesions together (Figs. 38.27, 38.28). This grouping has been used to differentiate predation by bed bugs from the typically single bites of other insects by the rule of three bites (breakfast, lunch and dinner), although memorable, is not diagnostic. Identification of the

insect is required to confirm the diagnosis. The final determination that bed bugs are involved depends on finding living or dead bugs or the circumstantial evidence of a characteristically pungent odor associated with the alarm glands of these insects and trails of blood droplets near the hiding places of the bugs.

Treatment

The itching associated with the bites of bed bugs responds to symptomatic therapy.

Control

Bed bugs typically begin their infestation near the sleeping areas and localize to mattress edges and beds. The populations can then expand to nearby locations including furniture and wall coverings. There has been a noted resurgence of this pest in many locations and control approaches have changed as well. DDT is now less commonly used or not used at all as it has been replaced with products containing a pyrethroid, a neonicotinoid or a combination of these two classes. These products are applied as an aerosol, powder, or liquid. Heat treatment is an option for those wanting to avoid chemical treatments. Vacuuming can have a significant impact on reducing the number of bed bugs. Laundering bedding and clothes and then running them through a hot dryer can kill bed bugs as well. Removal of old furniture, mattresses, and loose wallpaper, as well as the patching of wall cracks can deny the bugs resting and



Figure 38.30. Stinging Insects: hornet/wasp (*Vespa*), yellowjacket (*Vespula*), honey bee (*Apis*).

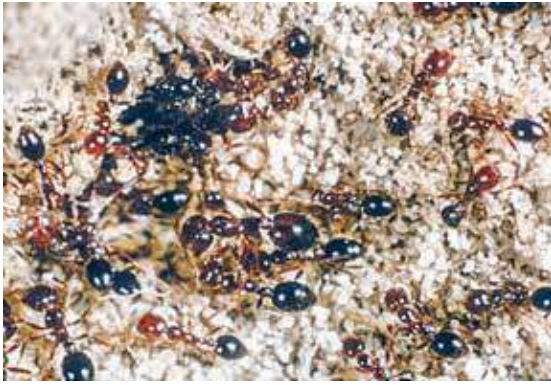


Figure 38.31. Fire ants, *Solenopsis invicta*.

breeding places. Mattress bags and monitoring can help insure that control methods have been effective.

Reduviidae: Assassin Bugs

The Reduviidae are a large family of predaceous insects collectively referred to as assassin bugs, most members of which are insectivorous. One subfamily, the Triatominae, is found mainly in the New World and is of particular importance because its members are hematophagous and are vectors of Chagas disease.

Bugs of the Triatominae (Fig. 38.29), the so-called kissing bugs, cone-nosed bugs, or *vinchucas*, are large insects with distinct elongate cone-shaped heads. They possess a long, three-segment proboscis that has been well developed for piercing skin; all developmental stages feed exclusively on blood. Adults are winged and are good fliers, whereas the nymphal stages are wingless. sexually

immature miniatures of the adults, developing through the various stages by incomplete metamorphosis (Fig. 38.1).

Historical Information

The Triatominae received little attention from entomologists before 1909, at which point Carlos Chagas identified them as the vectors of *Trypanosoma cruzi*.⁸⁰

Life Cycle

Three species of Triatominae serve as major vectors of Chagas disease, although several species are important as vectors in restricted areas and a large number of species have been found naturally infected with the parasite. *Panstrongylus megistus*, *Triatoma infestans*, and *Rhodnius prolixus* are large bugs that feed on humans, as well as on wild or domestic animals whenever they are available. These insects abound in the cracks and crevices of the mud and timber houses typical of rural areas in the Latin American tropics. Kissing bugs are usually found in resting places near their source of blood meals. Domestic or peridomestic species that prey on humans are found in cracks and crevices of walls and floors, particularly in sleeping areas.

Adult females require a blood meal before

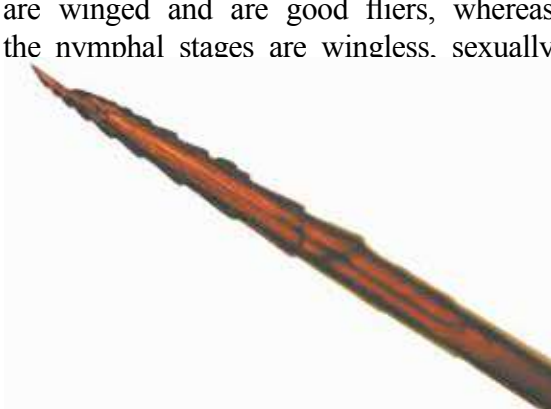


Figure 38.32. Bee stinger – note barbs.



Figure 38.33. Wasp stinger - barbs absent.

producing a clutch of eggs. They lay eggs singly within the same cracks that harbor the nymphs and adults. The eggs hatch within 10–30 days, and each of the five nymphal stages requires a full blood meal before molting to the next. Kissing bugs are prodigious feeders. First-stage nymphs can ingest 12 times their weight in blood; subsequent stages ingest relatively less. Fifth-stage nymphs of *R. prolixus* may ingest more than 300 μ l of blood, and adult females may ingest more than 200 μ l for each egg batch. The five nymphal stages may last several months before their final molt to the adult stage. Most species have one generation per year.

Pathogenesis

Intense, persistent pain is associated with the bites of some insectivorous assassin bugs, a result of injection of various toxins. The bites of most of the blood-feeding Triatominae are notably painless, enabling these insects to feed undisturbed on sleeping individuals. The habit of feeding around the mouth or eyes of a sleeper accounts for the designation “kissing bug.” After sensitization to the salivary fluids of feeding bugs, individuals can develop delayed reactions characterized by itching, swelling, redness, nausea, and, in rare cases, anaphylaxis. This serious response is a reported risk of xenodiagnosis, usually with *R. prolixus*.⁸¹

Both nymphs and adult bugs act as vectors of *T. cruzi*. Although bugs may be infected by feeding on human hosts and can transmit the organism from human to human, the usual sources of bug infections are wild and peridomestic animals (e.g., dogs, cats, mice, armadillos, and opossums). *T. cruzi* develops in the hindgut of the bug and does not invade the salivary glands. The bite of the bug does not cause infection. Rather, infectious parasites are passed in the feces of the bug while it feeds. The victim reacts to the irritation

of the bite, then rubs the infected feces into the eyes, mouth, or the wound made by the bite. Various triatomine species regularly feed on infected animals, supporting growth of the parasite, but do not defecate until they leave the host. These species usually are not involved in transmission to humans but may serve to maintain the parasite in an animal reservoir where infected bugs may be eaten by the animal host, which then becomes infected. *T. cruzi* is naturally maintained in wood rats in southern California by such a cycle. Although transmission of *T. cruzi* to humans is rare in the United States, infected animals are found regularly, and autochthonous cases have been reported.^{82–88}

Clinical Disease

Except for the allergic reaction to the bite, the bite itself is innocuous. The well recognized Romaña’s sign, a unilateral swelling around the eye, occurs when organisms are introduced into the body through mucous membranes due to a person rubbing them into their eye. This swelling is due to the infecting trypanosomes and not the saliva of the reduviid bug. The importance of these bugs lies in their acting as vectors in Chagas disease.

Diagnosis

The night-feeding kissing bugs are insidious, leaving little initial evidence of their blood meal. Kissing bugs normally feed from one puncture, leaving a single papule. This point distinguishes the lesion from those caused by bed bugs, which also feed at night but whose bites are usually clustered in groups of two or three.

Most of the entomophagous bugs likely to bite humans are large insects; the wheel bug, a common offender with a distinctive cog-like crest on its thorax, is more than 30 mm long. Because they bite during the day, usu-

ally when handled, they can be readily identified.

Treatment

Reactions to the bites of kissing bugs require, at most, local symptomatic therapy.

Control

Insecticides applied to houses have been effective for the control of some species of kissing bugs. Environmental control programs work better than even prudent and appropriate application of insecticides. For example, improved housing, in which thatched roofing and adobe walls and flooring are eliminated, helps to greatly reduce breeding sites for the bugs. Indoors, smooth walls with little in the way of pictures, etc. hanging on them also favors the elimination of Reduviidae from the local environment. These latter two approaches have been used in many parts of Brazil with a high degree of success. An aggressive vector control program now underway offers the promise of interruption of transmission of Chagas disease throughout most of South America.

Hymenoptera: The Stinging Insects

The stinging insects, including the bees, wasps, hornets (Fig. 38.30), and ants (Fig. 38.31), are members of the Hymenoptera, a large order of highly developed species. Complex social systems, castes, and elaborate hive and nest structures have evolved among the Hymenoptera. The only other insect group to achieve such a level of social development is the termite. In the Hymenoptera, the ovipositor (the apparatus used for egg laying) has been modified to serve as a stinging organ and is used by adult females to capture prey for food or for defense.

The stinging apparatuses of honey bees,

bumble bees, wasps, and hornets are generally similar in structure. They consist of paired acid glands, a single alkaline gland, a poison sac with muscular neck, and the piercing apparatus itself, which includes a pair of stylets and a stylet sheath. The stylets or darts of the honey bee stinger are barbed (Fig. 38.32) and, once inserted by the insect, cannot be withdrawn. Consequently, when a honey bee stings and attempts to fly away, it leaves behind the complete stinging apparatus, virtually disemboweling itself and suffering a mortal wound. The self-contained stinger with its attached poison sac and musculature continues to pump venom into the wound long after the bee has departed.^{89, 90} The stingers of most hymenopterans are not barbed (Fig. 39.33) and are withdrawn after stinging. Wasps, hornets, bumble bees, and ants are capable of multiple stings without losing their stinging apparatus.

It is estimated that in the United States 90–100 people die each year from reactions to stings of the hymenopterans, and even then, there is probably substantial underreporting and some misdiagnosis. Yellow jackets and honey bees are the major causes of such reactions.^{91, 92}

Certain behavioral characteristics of bees and yellowjackets result in increased aggressiveness. Honey bees are generally benign unless they are individually molested or provoked to defend their hive. Their venom contains the pheromone isopentyl acetate, which acts as an alarm signal and draws other bees to the site of the original sting, which in turn leads to multiple stings. Dramatic reports of the so-called African killer bees are often exaggerations of reality, although these bees tend to be more aggressive and less predictable than the common bees found in most domestic hives. The range of these “Africanized” honey bees has extended from South America through Central America and Mexico into the southern United States. The ultimate distribution into the United States will be limited by

the bees' ability to survive killing temperatures.⁹²⁻⁹⁴

Yellowjackets are particularly aggressive when their nest areas are approached, at which point they sting without provocation or warning. Their aggressive behavior increases during the late summer and early fall. Gardeners and picnickers are particularly at risk.

Historical Information

The honey bee, *Apis mellifera*, was one of the first insects recorded by humans in writings and art. Bees have long been recognized as sources of honey, and their role as plant pollinators is crucial to agriculture.

Life Cycle

Hymenopterans develop by complete metamorphosis with distinct larva, pupa, and adult stages. Larvae are vermiform and resemble maggots. They are dependent upon adults for food. Pupae, encased in a cocoon, represent an inactive, non-feeding transitional phase. The adults usually have wings and are good fliers. Certain groups with highly developed social systems have evolved non-reproducing worker and soldier castes. Other groups, such as the ants, are wingless as adults except during reproductive periods. Four families of the Hymenoptera contain medically important species, the stings of which can cause severe reactions in humans: Apidae, Vespidae, Formicidae, and Mutillidae.

The bees, or Apidae, include some species that live in complex social organizations such as hives or in less-structured subterranean nests, although most species in this family live as solitary insects. Only the honey bees and bumble bees among the Apidae are of concern to humans because of their ability to sting. The honey bee, *Apis mellifera*, origi-

nally an Old World species, is now found worldwide in domestic and wild hives. Bees of this species are raised commercially for their honey and for their role as pollinators of a wide range of plants, including most fruits and legume crops. They construct elaborate hives wherein a single non-foraging queen lays eggs in wax cells. Larvae develop within these cells while being fed by non-reproductive female workers. Adult workers tend the hives and forage for nectar and pollen. The bees tend to sting when their hive or individual insects are disturbed. Bumble bees of the genus *Bombus* are large, hairy, ungainly, less organized social bees that build simple underground nests. They sting under the same circumstances as do honey bees.

The Vespidae include the wasps, hornets, and yellowjackets, all of which are capable of inflicting painful stings. Many species in this family build elaborate nests of masticated wood fibers or mud, whereas others construct simple nests underground. The yellowjackets are social hymenopterans with distinctive yellow and black bands on the abdomen and are often mistaken for bees. They are aggressive insects and a major cause of stings in humans.

Ants belong to the family Formicidae, some members of which can cause damage by biting or stinging. They have a variety of complex social systems, with elaborate behavior patterns, intricate nests, and castes of workers, soldiers, and reproducing insects. Two groups of ants are of concern in the United States, the harvester ant and the fire ant.

The harvester ants of the genus *Pogonomyrmex* readily attack humans and other animals and are capable of inflicting painful stings. They build underground nests, topped by mounds, in warm, dry, sandy areas. When a nest is disturbed, ants come out and swarm over the invader; their stings are repeated and

vigorous.

Fire ants of the genus *Solenopsis* are so named because of their sharp, fiery sting. Several species native to the United States are of medical importance, but the imported fire ant, *Solenopsis invicta* (Fig. 38.31), is a particularly dangerous species.^{95,96} It was introduced into the United States around 1930 and since then has spread throughout the southeastern states, where it presents a serious hazard to humans and livestock. These ants build large, hard-crusted mounds, which are well camouflaged and often not seen until they are disturbed. When fire ants attack, they first bite their victim with strong mandibles, and then sting their victim repeatedly. The result is a circle of painful stings around a central bite.

Certain species of tropical ants are notorious for their ability to ravage plants and animals alike as they travel from place to place in colonies or armies numbering millions of individuals.

The so-called velvet ants are not true ants, but wingless wasps of the family Mutillidae. These large, hairy, often brightly colored insects are capable of inflicting a painful sting if they are disturbed. A large black mutillid with scarlet hairs is common in the central United States, where it can cause considerable distress by stinging barefoot bathers. Several other groups of the Hymenoptera have the capacity to sting.

Pathogenesis

During the act of stinging, the aroused insect first inserts the sheath, inflicting a wound, then follows immediately with the inward thrust of the stylets and injection of the venom. The combination of the acid and alkaline venom fluids, designed to kill insect prey, causes extreme pain and inflammation. Venom from 500 stings received within a few minutes can

cause death. The inhabitants of a single disturbed beehive can easily achieve that.

Sensitization to the venom can result in severe allergic reactions. A number of antigenically active compounds have been identified in venom, phospholipase A being the most important. Others include hyaluronidase, melittin, and apamin.^{97,98}

Clinical Disease

The primary manifestations of the sting are due to mechanical damage and the direct action of the venom. The pain, edema, pruritus, and warmth produced at the site of the sting are transitory. Severe toxic reactions can be caused by as few as 10 stings within a period of a few minutes. Muscle cramps, drowsiness, fever, and headache are characteristic.

Allergic reactions are by far the most serious consequence of the stings of hymenopterans. They may develop in previously sensitized individuals and include three symptom patterns: 1. urticaria associated with pruritus, 2. edematous skin and mucous membranes, and 3. simultaneous bronchospasm and anaphylaxis, followed sometimes by death. In sensitized individuals, even a single sting may bring the most severe reaction. A delayed reaction characterized by urticaria, fever, and arthralgia may occur hours or weeks after a sting.

Although the stinging hymenopterans share a number of common antigens, each possesses one or more unique antigens. Sensitization to stings of one species does not always produce sensitivity to those of other species.

Diagnosis

Individuals with suspected sting sensitivities can undergo skin testing with specific

venoms to determine the level of risk. Identification of the species posing the greatest threat to an individual may be critical.⁹⁷

Treatment

Initial treatment for a honey bee sting must include removal of its stinger and the attached venom sac. Removal can be accomplished with a knife blade, a needle, or a fingernail. It is important not to squeeze the site, because such pressure releases more venom from the sac. A non-allergic primary reaction may be treated with ice to lessen edema and pain, and with various local anti-pruritic compounds.

Individuals with known sting sensitivity must be prepared to act quickly to prevent serious reactions. Upon being stung, the individual should remove the stinger immediately. Emergency kits are available by prescription, and sensitive individuals should be familiar with their use. Such kits contain epinephrine in a syringe, antihistamine tablets, and a tourniquet. Emergency treatment consists of intramuscular injections of epinephrine and an antihistamine. Obviously, use of these

measures presupposes planning; the sensitized person and his or her next of kin must be prepared to carry out the intramuscular injection of epinephrine. In addition, the potential victim should carry an oral antihistamine medication such as diphenhydramine.

Desensitization using whole-body extracts of the insects has been attempted but is ineffective. Purified venoms have been used successfully for desensitization. Successful treatment for severe reactions to fire ant stings has been reported.

Control

Wasp, hornet, and ant nests can be destroyed with a number of commercially available insecticidal compounds, such as carbamates (also called urethranes), malathion (an organophosphate), and resmethrin (a pyrethroid). Aerial nests can be destroyed at night, when the insects are quiescent. General avoidance of areas where stinging hymenopterans occur should be a rule for sensitive individuals. There are no effective repellents against these insects.

References

1. Savely, V. R., et al., The mystery of Morgellons disease: infection or delusion? *Am J Clin Dermatol* **2006**, 7 (1), 1-5.
2. Koo, J.; Lee, C. S., Delusions of parasitosis. A dermatologist's guide to diagnosis and treatment. *Am J Clin Dermatol* **2001**, 2 (5), 285-90.
3. Ozkan, A. T.; Mumcuoglu, K. Y., [Entomophobia and delusional parasitosis]. *Turkiye Parazitoloj Derg* **2008**, 32 (4), 366-70.
4. Peng, Z., et al., Mosquito allergy and mosquito salivary allergens. *Protein Pept Lett* **2007**, 14 (10), 975-81.
5. Ribeiro, J. M. C.; Francischetti, I. M. B., Role of arthropod saliva in blood feeding: Sialome and post-sialome perspectives. *Annu Rev Entomol* **2002**, 48, 73-88.
6. Theodorides, J., Note historique sur la decouverte de la transmission de la leishmaniose cutanee par les phlebotomes. *Bull Soc Pathol Exot* **1997**, 90, 177-180.
7. Oliveira, F., et al., A sand fly salivary protein vaccine shows efficacy against vector-transmitted cutaneous leishmaniasis in nonhuman primates. *Sci Transl Med* **2015**, 7 (290), 290ra90.
8. Elnaïem, D. E., et al., Genetic variation in the sand fly salivary protein, SP-15, a potential vaccine candidate against *Leishmania major*. *Insect Mol Biol* **2005**, 14 (2), 145-50.
9. Wenyon, C. M.; J., Some recent advances in our knowledge of leishmaniasis. *School Trop Med* **1912**, 1 93-98.
10. Lewis, D. J., The biology of Phlebotomidae in relation to leishmaniasis. *Annual review of*

- entomology* **1974**, *19*, 363-84.
11. Valenzuela, J. G., et al., Identification of the most abundant secreted proteins from the salivary glands of the sand fly *Lutzomyia longipalpis*, vector of *Leishmania chagasi*. *The Journal of experimental biology* **2004**, *207* (Pt 21), 3717-29.
12. Sambon, L. W.; J., Progress report of investigations of pellagra. *Med* **1910**, *13* 271-287.
13. Monnerat, R., et al., Synergistic activity of *Bacillus thuringiensis* toxins against *Simulium* spp. larvae. *J Invertebr Pathol* **2014**, *121*, 70-3.
14. To, K. K.; Yuen, K. Y., In memory of Patrick Manson, founding father of tropical medicine and the discovery of vector-borne infections. *Emerg Microbes Infect* **2012**, *1* (10), e31.
15. Sonenshine, D. E., *Biology of Ticks Volume 2*. **2013**.
16. de Kruif, P., *The Microbe Hunters*. **1926**.
17. Harrison, G., *Mosquitos, Malaria, and Man*. **1978**.
18. Shah, S., The Fever: How malaria has ruled mankind for 500,000 years. **2010**.
19. Reed, W., et al., Etiology of yellow fever, a preliminary note. *Philos* **1900**, *6*, 790-796.
20. Reinert, J. F., New classification for the composite genus *Aedes* (Diptera: Culicidae: Aedini), elevation of subgenus *Ochlerotatus* to generic rank, reclassification of the other subgenera, and notes on certain subgenera and species. *Journal of the American Mosquito Control Association* **2000**, *16* (3), 175-88.
21. Reinert, J. F., et al., Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. *Zool Soc* **2004**, *142* 289-368.
22. Singh, S.; Mann, B. K., Insect bite reactions. *Indian J Dermatol Venereol Leprol* **2013**, *79* (2), 151-64.
23. Morsy, T. A., Insect bites and what is eating you? *J Egypt Soc Parasitol* **2012**, *42* (2), 291-308.
24. Williams, L. A.; Allen, L. V., Jr., Treatment and prevention of insect bites: mosquitoes. *Int J Pharm Compd* **2012**, *16* (3), 210-8.
25. Steen, C. J., et al., Arthropods in dermatology. *J Am Acad Dermatol* **2004**, *50* (6), 819-42, quiz 842-4.
26. Villar, L., et al., Efficacy of a tetravalent dengue vaccine in children in Latin America. *N Engl J Med* **2015**, *372* (2), 113-23.
27. McArthur, M. A., et al., Dengue vaccines: recent developments, ongoing challenges and current candidates. *Expert Rev Vaccines* **2013**, *12* (8), 933-53.
28. Kendrick, K., et al., Notes from the field: Transmission of chikungunya virus in the continental United States--Florida, 2014. *MMWR Morb Mortal Wkly Rep* **2014**, *63* (48), 1137.
29. Winskill, P., et al., Dispersal of Engineered Male *Aedes aegypti* Mosquitoes. *PLoS Negl Trop Dis* **2015**, *9* (11), e0004156.
30. Allan, S. A., Susceptibility of adult mosquitoes to insecticides in aqueous sucrose baits. *J Vector Ecol* **2011**, *36* (1), 59-67.
31. Leiper, R. T., Report of the Helminthologist for the Half Year Ending 30 April, . Report of the Advisory Commission on Tropical Diseases Research Fund. **1913**, 1913-1914.
32. Benecke, M., A brief history of forensic entomology. *Forensic Sci Int* **2001**, *120* (1-2), 2-14.
33. Michaud, J. P., et al., Rewriting Ecological Succession History: Did Carrion Ecologists Get There First? *Q Rev Biol* **2015**, *90* (1), 45-66.
34. Amendt, J., et al., Forensic entomology: applications and limitations. *Forensic Sci Med Pathol* **2011**, *7* (4), 379-92.
35. Scudder, H. I.; Linda, D. R., Nonbiting flies and disease *Annu Rev Entomol* **1956**, *1*, 323-346.
36. Greenberg, B., *Flies and Disease*. 1973; Vol. II.
37. Franco, J. R., et al., Epidemiology of human African trypanosomiasis. *Clin Epidemiol* **2014**, *6*, 257-75.
38. Aksoy, S., et al., Interactions between tsetse and trypanosomes with implications for the control of trypanosomiasis. *Adv Parasitol* **2003**, *53*, 1-83.
39. Francesconi, F.; Lupi, O., Myiasis. *Clin Microbiol Rev* **2012**, *25* (1), 79-105.
40. Morley, W. N., Body infestations. *Scottish medical journal* **1977**, *22* (3), 211-6.
41. Steenvoorde, P., et al., Maggot debridement therapy of infected ulcers: patient and wound factors influencing outcome - a study on 101 patients with 117 wounds. *Ann R Coll Surg Engl* **2007**, *89*

- (6), 596-602.
42. Sun, X., et al., A systematic review of maggot debridement therapy for chronically infected wounds and ulcers. *Int J Infect Dis* **2014**, *25*, 32-7.
43. Mumcuoglu, K. Y., et al., Pain related to maggot debridement therapy. *J Wound Care* **2012**, *21* (8), 400, 402, 404-5.
44. Gilead, L., et al., The use of maggot debridement therapy in the treatment of chronic wounds in hospitalised and ambulatory patients. *J Wound Care* **2012**, *21* (2), 78, 80, 82-85.
45. Whitaker, I. S., et al., Larval therapy from antiquity to the present day: mechanisms of action, clinical applications and future potential. *Postgrad Med J* **2007**, *83* (980), 409-13.
46. Gregory, A. R., et al., Ophthalmomyiasis caused by the sheep bot fly *Oestrus ovis* in northern Iraq. *Optom Vis Sci* **2004**, *81* (8), 586-90.
47. Lord, W. D., et al., Collection and preservation of forensically important entomological materials. *Science* **1983**, *28*, 936-944.
48. Greenberg, B., Forensic entomology: case studies. *Bull Entomol Soc Am* **1985**, *31*, 25-28.
49. Greenberg, B.; Kunich, J. C., Entomology and the law. **2002**.
50. Chosidow, O., Scabies and pediculosis. *Lancet (London, England)* **2000**, *355* (9206), 819-26.
51. Zinsser, H., Rats, Lice and History. 1935.
52. Busvine, J. R., Insects, Hygiene, and History. *Athlone Press London* **1976**.
53. Do-Pham, G., et al., Lice. *Semin Cutan Med Surg* **2014**, *33* (3), 116-8.
54. Ko, C. J.; Elston, D. M., Pediculosis. *J Am Acad Dermatol* **2004**, *50* (1), 1-12; quiz 13-4.
55. Leone, P. A., Scabies and pediculosis pubis: an update of treatment regimens and general review. *Clin Infect Dis* **2007**, *44 Suppl 3*, S153-9.
56. Roberts, R. J., Clinical practice. Head lice. *N Engl J Med* **2002**, *346* (21), 1645-50.
57. Osorio, C., et al., Plica polonica secondary to seborrheic dermatitis. *J Eur Acad Dermatol Venereol* **2015**.
58. Nara, A., et al., An unusual autopsy case of lethal hypothermia exacerbated by body lice-induced severe anemia. *Int J Legal Med* **2015**.
59. Jahnke, C., et al., Accuracy of diagnosis of pediculosis capitis: visual inspection vs wet combing. *Arch Dermatol* **2009**, *145* (3), 309-13.
60. Ackerman, A. B., Crabs--the resurgence of Phthirus pubis. *N Engl J Med* **1968**, *278* (17), 950-1.
61. Feldmeier, H., Treatment of pediculosis capitis: a critical appraisal of the current literature. *Am J Clin Dermatol* **2014**, *15* (5), 401-12.
62. Burkhart, C. G.; Burkhart, C. N., Oral ivermectin for Phthirus pubis. *J Am Acad Dermatol* **2004**, *51* (6), 1037; author reply 1037-8.
63. Bohl, B., et al., Clinical Practice Update: Pediculosis Capitis. *Pediatr Nurs* **2015**, *41* (5), 227-34.
64. Walsh, J.; Nicholson, A., Head lice in children--a modern pandemic. *Ir Med J* **2005**, *98* (5), 156-7.
65. Elston, D. M., Treating pediculosis--those nit-picking details. *Pediatr Dermatol* **2007**, *24* (4), 415-6.
66. Deeks, L. S., et al., Topical ivermectin 0.5% lotion for treatment of head lice. *Ann Pharmacother* **2013**, *47* (9), 1161-7.
67. Orion, E., et al., Ectoparasitic sexually transmitted diseases: scabies and pediculosis. *Clinics in dermatology* **2004**, *22* (6), 513-9.
68. Elgart, M. L., Current treatment for scabies and pediculosis. *Skin Therapy Lett* **2000**, *5*, 1-3.
69. Raoult, D.; Roux, V., The body louse as a vector of reemerging human diseases. *Clin Inf Dis* **1999**, *29* (4), 888-911.
70. Palicelli, A., et al., Tungiasis in Italy: An imported case of *Tunga penetrans* and review of the literature. *Pathol Res Pract* **2016**.
71. Feldmeier, H., et al., Tungiasis (sand flea disease): a parasitic disease with particular challenges for public health. *Eur J Clin Microbiol Infect Dis* **2013**, *32* (1), 19-26.
72. Feldmeier, H.; Keyzers, A., Tungiasis - A Janus-faced parasitic skin disease. *Travel Med Infect Dis* **2013**, *11* (6), 357-65.

73. Grunwald, M. H., et al., Tungiasis. *The Australasian journal of dermatology* **2000**, 41 (1), 46-7.
74. Heukelbach, J., Revision on tungiasis: treatment options and prevention. *Expert Rev Anti Infect Ther* **2006**, 4 (1), 151-7.
75. Huntley, A. C., *Cimex lectularius*. What is this insect and how does it affect man? *Dermatology online journal* **1999**, 5 (1), 6.
76. Kolb, A., et al., Bedbugs. *Dermatol Ther* **2009**, 22 (4), 347-52.
77. Simond, M., et al., Paul-Louis Simond and his discovery of plague transmission by rat fleas: a centenary. *J R Soc Med* **1998**, 91 (2), 101-4.
78. Farhang-Azad, A., et al., Transovarial transmission of murine *Typhus rickettsiae* in *Xenopsylla cheopis* fleas. *Science (New York, N.Y.)* **1985**, 227 (4686), 543-5.
79. Jupp, P. G., et al., Attempts to transmit hepatitis B virus to chimpanzees by arthropods. *South African medical journal (Suid-Afrikaanse tydskrif vir geneeskunde)* **1991**, 79 (6), 320-2.
80. Chagas, C., Nova trypanozomíase humana: estudos sobre a morfologia e o ciclo evolutivo do *Schizotrypanum cruzi*. n genl n sp agente etiológico de nova entidade morbida do homem. *Mem Inst Oswaldo Cruz* **1909**, 1, 159-218.
81. Costa, C. H., et al., Skin reactions to bug bites as a result of xenodiagnosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1981**, 75 (3), 405-8.
82. Morel, C. M., Chagas' disease, from discovery to control and beyond: history, myths and lessons to take home. *Mem Inst Oswaldo Cruz* 94 Suppl **1999**, 1 3-16.
83. Ebrahim, G. J., Eradication of American trypanosomiasis (Chagas' disease): an achievable goal? *Journal of tropical pediatrics* **2004**, 50 (6), 320-1.
84. Klotz, S. A., et al., Kissing bugs in the United States: risk for vector-borne disease in humans. *Environ Health Insights* **2014**, 8 (Suppl 2), 49-59.
85. Bern, C., et al., *Trypanosoma cruzi* and Chagas' Disease in the United States. *Clin Microbiol Rev* **2011**, 24 (4), 655-81.
86. Garcia, M. N., et al., Historical Perspectives on the Epidemiology of Human Chagas Disease in Texas and Recommendations for Enhanced Understanding of Clinical Chagas Disease in the Southern United States. *PLoS Negl Trop Dis* **2015**, 9 (11), e0003981.
87. Stevens, L., et al., Kissing bugs. The vectors of Chagas. *Adv Parasitol* **2011**, 75, 169-92.
88. Klotz, J. H., et al., "Kissing bugs": potential disease vectors and cause of anaphylaxis. *Clin Infect Dis* **2010**, 50 (12), 1629-34.
89. Tankersley, M. S.; Ledford, D. K., Stinging insect allergy: state of the art 2015. *J Allergy Clin Immunol Pract* **2015**, 3 (3), 315-22; quiz 323.
90. Touchard, A., et al., The Biochemical Toxin Arsenal from Ant Venoms. *Toxins (Basel)* **2016**, 8 (1).
91. Fitzgerald, K. T.; Flood, A. A., Hymenoptera stings. *Clin Tech Small Anim Pract* **2006**, 21 (4), 194-204.
92. Potiwat, R.; Sitcharungsi, R., Ant allergens and hypersensitivity reactions in response to ant stings. *Asian Pac J Allergy Immunol* **2015**, 33 (4), 267-75.
93. Shaker, M. S., et al., An update on venom allergy. *Curr Opin Pediatr* **2013**, 25 (5), 629-34.
94. Winston, M. L., The biology and management of Africanized honey bees *Entomol* 547 **1992**, 37 S, 173-193.
95. Caplan, E. L., et al., Fire ants represent an important risk for anaphylaxis among residents of an endemic region. *The Journal of allergy and clinical immunology* **2003**, 111 (6), 1274-7.
96. Stafford, C. T., Hypersensitivity to fire ant venom. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology* **1996**, 77 (2), 87-95; quiz 96.
97. Wong, H. C., Importance of proper identification of stinging insects. *Annals of internal medicine* **2000**, 132 (5), 418.
98. Hoffman, D. R., Insect venom allergy, immunology and immunotherapy. 1984; p 187-223.



Miriam Rothschild (1908–2005)

During her life, Miriam Rothschild was the world's leading authority on fleas. In addition to being the first person to elucidate the jumping mechanism of fleas, she published on the role of *Xenopsylla cheopsis* as a vector for plague. Rothschild went on to publish dozens of papers on the digenetic trematodes and even after her laboratory and data were destroyed by a Nazi attack she went on to write the book *Fleas, Flukes, and Cuckoos*, which was written in such a manner as to make parasitology accessible to the general reader. By the time of her death in 2005, she had authored over 300 papers and several texts.

39. Arachnids

Introduction

The arachnids comprise a class of arthropods that includes the ticks, mites, scorpions, and spiders. The characteristics of the Arachnida clearly differentiate it from the class Insecta. All arachnids are wingless, have four pairs of legs as adults, and usually show only two distinct body regions: a cephalothorax and an abdomen. Metamorphosis among the arachnids is of the incomplete type. The immature, non-reproductive stages are smaller but morphologically similar to the adults. In many groups, arachnids in the first, or larval, stage may have only three pairs of legs.

Arachnida is comprised of 11 extant orders, 3 of which are Acari, Araneae, and Scorpiones. The order Acari includes mites and ticks. Ticks are exclusively hematophagous, whereas mites feed on a variety of substances, including insect eggs, cells and blood. The spiders (order Araneae) are mainly insectivorous, feeding on body fluids of captured insects. Some larger tarantulas may feed on small mammals or birds. Scorpions (order Scorpiones) feed on arthropods or small animals that they have immobilized with their stinging apparatus, which is located at the tip of the tail.

Most members of these three orders do not affect human health directly. Each order includes some members of medical importance.

Acari (Ticks and Mites)

Ticks

The ticks comprise two large families: the Ixodidae (hard ticks) and the Argasidae (soft ticks). Ticks are responsible for damage to livestock, causing considerable weight loss, and for providing opportunities for secondary infection by bacteria. Many species are capable of transmitting pathogens to domestic animals and humans. The salivary secretions of some species can cause paralysis (tick paralysis) and even death in humans or other mammals.

Ticks and mites injure their victims by their feeding habits and serve as vectors for a number of important diseases (Table 39.1). The consequences of infestations by ticks are enormous in terms of yearly losses in dairy and meat production. In areas of the world where sources of protein are already scarce, tick infestations have created a crisis situation. Humans are seldom the natural host for any species of tick. Many species will feed on human blood and have the opportunity to become vectors of infections.

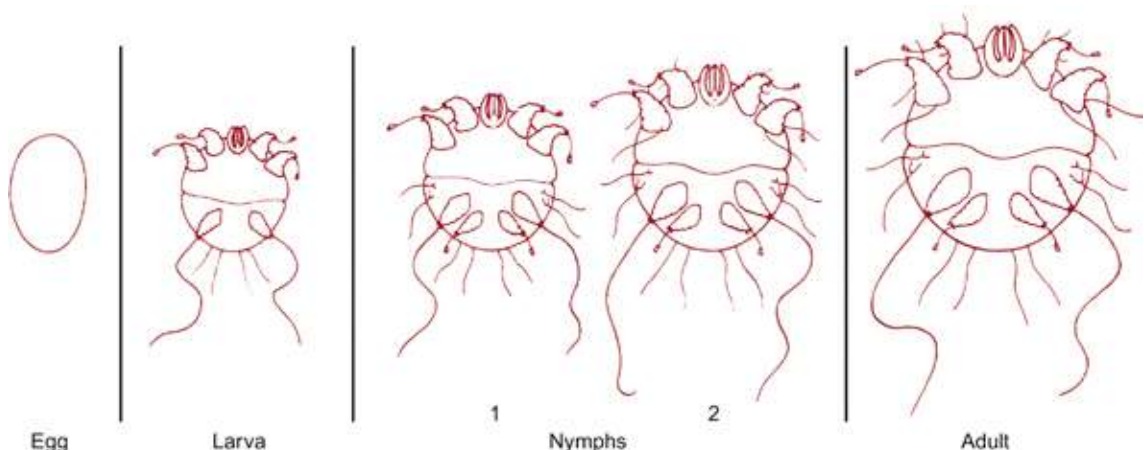


Figure 39.1. Incomplete metamorphosis in the arachnida. Ticks and mites undergo incomplete metamorphosis as typified by the itch mite, *Sarcoptes scabiei*. Larvae have three pair of legs; adults and nymphal stages have four pairs of legs.

Homer recorded the feeding of ticks on humans during the ninth century BCE, as did Aristotle during the fourth century BCE. One of the earliest references to ticks as a possible cause of disease was the suggestion by a 12th-century Persian physician that a fever (probably Crimean-Congo hemorrhagic fever) was transmitted by ticks.¹

Theobald Smith and Frederick L. Kilbourne were the first to demonstrate that ticks could transmit disease.² They reported that the tick *Boophilus annulatus* carried the bovine protozoan parasite *Babesia bigemina*, a serious pathogen of cattle in the western United States. They further demonstrated that a single infected tick did not pass the parasite from cow to cow; rather, it was transmitted from an infected cow through a female tick to the tick's offspring, transovarially. This mechanism, referred to as vertical transmission, resulted in infection of larval ticks capable of transmitting the parasite at the time of their first feeding. These authors reported their findings in 1893, four years before Ronald Ross completed his studies on the transmission of malaria by mosquitoes. The role of ticks as vectors of spirochetes was shown first with an avian parasite by Émile Marchoux and Alexandre Salimbeni in 1903. A year later a spirochete was demonstrated to cause human relapsing fever by Ronald Ross and A.D. Milne.^{3,4}

Hard Ticks: Family Ixodidae

Hard ticks (Fig 39.2, family Ixodidae) are found throughout the world as ectoparasites of a variety of animals. Their name derives from the characteristic tough, leather-like integument that covers most of their body. Their mouthparts are included in a capitulum (Fig. 39.2), but there is no defined head. Members of both sexes feed exclusively on blood.

The typical hard tick develops by gradual



Figure 39.2. Deer tick, *Ixodes scapularis*. Photo D. Scharf.

metamorphosis from the egg through the larva and nymph to the adult. Larvae have three pairs of legs; the nymphs and adults have four pairs. Each stage takes a single blood meal. The larvae and nymphs feed prior to molting, and the adult females feed prior to producing a single batch of eggs. The female tick dies after oviposition.⁵

Hard ticks exhibit one of three life cycles and may be classified as one-, two-, or three-host ticks. A one-host tick spends its life on a single animal. It attaches to the skin of its host as a larva, feeds, and then molts to the nymph stage. After feeding again, it molts a second time, developing into the adult. The adults mate, after which the female, engorged with blood, falls to the ground, and lays her eggs. Larvae begin to hatch within 30 days, and await a new host to begin the cycle again.

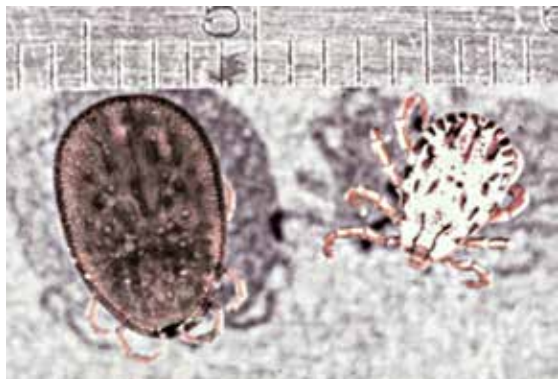


Figure 39.3. Soft (left) and hard ticks (right).

Table 39.1. Arthropods of medical importance.

Order and representative species	Common name	Geographic distribution	Effects on humans
Acari (ticks and mites)			
Argasidae: various genera and species	Soft tick	Worldwide	Skin reactions to bite, tick paralysis, vectors of relapsing fever
Ixodidae: various genera and species	Hard tick	Worldwide	Skin reactions to bite; tick paralysis; vectors of rickettsia, viruses, bacteria, and protozoa
Dermanyssidae <i>Liponyssoides sanguines</i>	House mouse mite	Worldwide	Vector of <i>Rickettsia akari</i> , the cause of rickettsial pox
Various other genera	Mite	Worldwide	Occasional dermatitis from bite
Demodicidae <i>Demodex folliculorum</i>	Follicle mite	Worldwide	Found in sebaceous glands and hair follicles Occasional skin reactions
Trombiculidae <i>Trombicula</i> spp. <i>Leptotrombidium palpalis</i>	Harvest mite Mite	Worldwide Southeast Asia, India, Pacific Islands	Intense itching at site of attachment Vector of <i>Orientia tsutsugamushi</i> , cause of scrub typhus
Sarcoptidae <i>Sarcoptes scabiei</i>	Human itch mite	Worldwide	Burrows in skin causing severe itching
Araneae (spiders)			
<i>Latrodectus</i> spp. <i>Loxocles</i> spp.	Widow spider Recluse spider	Worldwide Americas	Bite painless; delayed systemic reaction Initial blister, then necrosis with slow healing
Scorpiones (scorpions)			
Various genera	Scorpion	Tropics/subtropics	Initial painful sting then systemic reactions

Two-host ticks usually spend their larva and nymph stages on one host, drop to the ground, molt, and await a second host of another species for completion of the adult phase of the cycle. Each of the three stages of a three-host tick develops on a separate host. The immature stages are usually found on small rodents. The adults feed and mate on larger animals.

Hard ticks display remarkable longevity, with adults of many species surviving up to two years without a blood meal. One-host ticks have the shortest egg-to-egg life cycles, sometimes lasting less than a year. Three-host ticks require 2–3 years to complete their life cycles.

Hard ticks feed slowly, requiring 7–9 days to become completely engorged. After attaching to a suitable host, the tick searches for a feeding site often well concealed by hair. Once in place, it inserts its mouthparts armed with

re-curved teeth, secretes a cement-like substance, and begins to feed. After engorging it easily detaches and moves away. In general, the act of feeding is painless to the host, who is often unaware of the tick.^{5,6}

There are 11 genera within the Ixodidae, some of which include species of ticks that feed on humans, and so are of medical importance. *Amblyomma americanum* and *A. cajennense* prey on a variety of animals, feed avidly on humans, and are serious pests in the southern and southwestern states of the United States and in Mexico. They are capable of transmitting the rickettsiae that cause Rocky Mountain spotted fever (*Rickettsia rickettsii*). *A. americanum* can also transmit southern tick-associated rash illness (STARI).

Dermacentor variabilis (Fig. 39.4), the American dog tick, is the major vector of Rocky Mountain spotted fever in the eastern



Figure 39.4. Blood-engorged adult female American dog tick, *Dermacentor variabilis*. Courtesy W. Burgdorfer.

and central United States. It is involved in the transmission of tularemia and can cause tick paralysis in humans and dogs. *D. variabilis* is a three-host tick. The larvae and nymphs feed on small rodents, and the adults feed and mate on larger mammals. The dog is the most common host for adults of this species, but humans are readily targeted as well.

D. andersoni (Fig. 39.5), the Rocky Mountain wood tick, is a common species in the western and northern United States. It transmits Rocky Mountain spotted fever and Colorado tick fever (Colorado tick fever virus), and it causes tick paralysis in humans. This



Figure 39.5. *Ixodes scapularis*. The adult feeds on deer. Nymphs transmit spirochetes and *Babesia* to humans.

three-host tick feeds on a variety of small mammals as a larva or nymph. As an adult, it feeds on large wild or domestic animals and humans. Both nymphs and adults are capable of over-wintering, and the life cycle of this species is usually greater than two years. *D. albipictus* and *D. occidentalis*, found in the western United States, are capable of transmitting Rocky Mountain spotted fever and Colorado tick fever, but they attack humans only infrequently.⁵⁻¹¹

Ixodes scapularis (Fig. 39.6, black-legged tick or deer tick) is a three-host tick common throughout the eastern United States. It readily attacks humans and can inflict painful bites. In New England, it had been suggested that a distinct species, *I. dammini*, was responsible for the transmission of human babesiosis and later for being the vector of Lyme disease.^{7,8} Subsequent studies determined that a single species, *I. scapularis*, was involved throughout the area.⁹ *I. pacificus* is a common pest of deer and cattle in California. It readily bites humans as well, and has been implicated in the transmission of the Lyme spirochete (*Borrelia burgdorferi*).¹⁰ *I. holocyclus* is an important cause of tick paralysis in Australia.



Figure 39.6. *Dermacentor andersoni*. Adult female on vegetation, awaiting a host. Courtesy W. Burgdorfer.

Rhipicephalus sanguineus, the brown dog tick, is a cosmopolitan ectoparasite of dogs. Although this species does not readily bite humans, it can be a serious nuisance around homes. Female ticks recently engorged on the blood of domestic dogs drop off and deposit eggs in houses or kennels. The newly hatched larvae tend to crawl up vertical surfaces, literally covering walls or furniture. *R. sanguineus* is considered the major vector of the rickettsia that causes boutonneuse fever (*Rickettsia conorii*).

Hyalomma and *Boophilus* are genera of ticks whose members are ectoparasites of animals and play important roles in transmitting pathogens in animal populations. Occasionally, these ticks act as vectors of human diseases.

Soft Ticks: Family Argasidae

Ticks of the family Argasidae are soft-bodied arthropods covered by a wrinkled, often granulated tegument (Fig. 39.3). They do not have a distinct head region, and their mouthparts are located on the ventral surface, not visible from above. Soft ticks are found throughout the world, usually as ectoparasites of birds, although some species normally feed on bats and other small mammals. Several species attack humans if given the opportunity.

Soft ticks differ from the hard ticks in their feeding behavior, habitat, and life cycles. Soft ticks normally inhabit the nesting site of their hosts, moving onto the host to feed and returning to the nest when finished. They are completely engorged within a matter of minutes or a few hours at most, usually feeding at night while the host is asleep.

The typical life cycle of a soft tick consists of a single six-legged larval stage, two or more eight-legged nymph stages, and the eight-legged adult stage. Some species require sev-

eral blood meals before each molt, and adult females feed repeatedly, producing a small batch of eggs after each meal.

Three genera of soft ticks affect humans as pests or as vectors of pathogens. The fowl tick *Argas persicus* is an important cosmopolitan ectoparasite that preys on poultry. It bites humans as well, particularly if the normal fowl hosts are unavailable. Ticks of the genus *Otobius* occasionally infest the ears of humans.

Ticks of the genus *Ornithodoros* are important pests and vectors of the spirochetes responsible for tick-borne relapsing fevers. *O. moubata* attacks a number of wild and domestic animals, but humans are its major host. This tick inhabits huts, feeding at night on the sleeping inhabitants. It is found throughout southern and central Africa reaching as far north as Ethiopia, and it is the major vector of African relapsing fever caused by various *Borrelia* spp. Epidemiologic evidence suggests that *O. moubata*, the tampan tick, may be involved in the transmission of hepatitis B virus in Africa, but direct experimental evidence is lacking.¹¹

Several species of *Ornithodoros* are vectors of relapsing fevers in both the New World and the Old World. Most of these species, however, are ectoparasites of rodents and other mammals, feeding on humans only occasionally.

Pathogenesis and Treatment of Tick Bites

Most ticks attach themselves firmly to the skin of the host before beginning the blood meal. The mouthparts and injected salivary secretions provoke inflammation of the surrounding tissue, characterized by local hyperemia, edema, hemorrhage, and thickening of

the stratum corneum.¹² Although the initial bite and insertion of the mouthparts may be painless, irritation often develops later, followed by necrosis and secondary infection at the wound site.

It is important to remove ticks from the skin of the host as soon as they are detected, preventing firm attachment and making transmission of pathogens less likely. It also limits the infusion of toxins that cause tick paralysis, as these toxins are released slowly.

Numerous methods have been suggested for the removal of firmly attached ticks. Traditionally, ticks have been treated with chloroform, ether, benzene, turpentine, or petroleum jelly, each of which, it has been suggested, irritates the tick, causing it to withdraw. The U.S. Public Health Service recommends mechanical removal without chemical aids. Many genera of ticks, especially *Dermacentor*, may be removed by gently but firmly, pulling the tick away from its attachment. *Ixodes* and *Amblyomma*, which have longer mouthparts that do not detach easily, may require the use of instruments for removal. These ticks should be pulled gently away from the host so the skin surrounding the mouthparts forms a tent. Use a pair of forceps or tweezers rather than a knife blade. In all cases, care should be taken to avoid leaving any tissue from the tick, as it will induce intense inflammation. The tick should not be crushed or damaged, thereby preventing the release of pathogenic organisms onto the wound site. Subsequent thorough cleaning of the wound is recommended.¹³

Tick Paralysis

More than 40 species in 10 genera of both hard and soft ticks secrete salivary toxins that cause paralysis in humans and a number of other animals. It is not a universal property of any one species, though, suggesting that salivary secretions are characteristic of individual ticks.¹⁴⁻¹⁸

The affected patient becomes irritable, is restless, and experiences numbness and tingling in the extremities, face, lips, and throat. Soon, the patient develops symmetric, flaccid paralysis that is ascending in nature and can lead to bulbar palsy. Sensory loss is rare. There is no fever. Death results from respiratory paralysis. The laboratory findings (complete blood count, urinalysis, and CSF examination) are normal. Differential diagnosis includes poliomyelitis, Guillain-Barré syndrome, transverse myelitis, and spinal cord tumors. The diagnosis depends on the patient's clinical history and finding the tick. Treatment consists in removing the feeding tick. Recovery follows rapidly. The usual causes of human tick paralysis are *D. andersoni* and *D. variabilis* in the United States and *I. holocyclus* in Australia. A number of species of *Dermacentor*, *Ixodes*, *Amblyomma*, *Rhipicephalus*, *Argas*, and *Ornithodoros* often cause paralysis in animals, but only occasionally affect humans. A vaccine for tick paralysis is under development.¹⁹⁻²³

Tick-Borne Diseases in Humans

Ticks transmit a broad array of viruses, rickettsiae, bacteria, and protozoa, which may cause disease in their human hosts.

Viral Diseases

Colorado tick fever is the most common tick-borne viral disease in humans in the United States. An often-benign disease transmitted by the bite of *D. andersoni*, it is maintained in nature as an enzootic infection of rodents spread by the same vector. Transovarial (vertical) transmission of the virus (Colorado tick fever virus) has not been demonstrated. Colorado tick fever is characterized by sudden onset of chills, headache, severe myalgia, and fever.²⁴

In the Old World, hard ticks are vectors of a number of viral diseases grouped as hemorrhagic fevers or tick-borne encephalitides. Among them are Russian spring-sum-

mer encephalitis, Kyasanur Forest disease, Crimean-Congo hemorrhagic fever, and Omsk hemorrhagic fever.

Rickettsial Diseases

Rocky Mountain spotted fever is an acute, sometimes fatal, febrile, exanthematous disease caused by *Rickettsia rickettsii*. It most frequently affects children and is characterized by fever, headache, musculoskeletal pain, and a generalized rash that appears first on the wrists and ankles, and often becomes hemorrhagic.

Although initially described from the Rocky Mountain region of the United States and distributed throughout much of North and South America, the infection is of particular importance in the “tick belt” states of Maryland, Virginia, North Carolina, South Carolina, and Georgia. In these states, the incidence of the disease has been rising steadily. The main vector in the eastern United States is *D. variabilis*, the American dog tick; in the western states it is *D. andersoni*. Other tick species that are considered minor vectors have the capacity to transmit the organism to humans but may be primarily important as vectors that infect reservoir hosts.

In areas where Rocky Mountain spotted fever is prevalent, regular inspection for ticks should be undertaken. Children especially should be examined twice daily. The tick must be attached to the host for several hours before it transmits the pathogen; therefore, its expeditious removal can prevent infection. No vaccine against Rocky Mountain spotted fever is currently available, but infections can easily be treated with doxycycline. Untreated cases have a mortality rate of 2–5%.^{13, 24, 25}

Old World tick-borne typhus has different regional names: boutonneuse fever, Kenya typhus, and South African tick bite fever. It is a relatively mild disease, presenting with chills, fever, and generalized body rash.

Rhipicephalus sanguineus, the brown dog tick, is the main vector of boutonneuse fever in the Mediterranean region; other hard ticks are involved elsewhere.

Q fever, or query fever is not always a self-limited infection. The disease consists of fever, headache, constitutional symptoms, and often pneumonitis. Caused by the rickettsia *Coxiella burnetii*, it is usually contracted by inhalation. Ticks are involved in maintaining the infection in the animal reservoir host and can transmit the organism to humans.

Anaplasmosis is a tick-borne rickettsiosis, first described in Japan in 1954, that resembles other tick-borne diseases such as “spotless” Rocky Mountain spotted fever. This generally mild infection may be mistaken for pyelonephritis, hepatitis C or D, gastroenteritis, or unexplained febrile illnesses with leukopenia or thrombocytopenia. Treatment is similar to that for other rickettsial diseases.²⁶
²⁷ Co-infection with more than one tick-borne pathogen in a single individual has been reported.²⁸

Bacterial Diseases

Tularemia is a bacterial disease caused by *Francisella tularensis* and characterized by a focal ulcer at the site of entry of the organism, enlargement of regional lymph nodes, fever, prostration, myalgia, and headache. *Dermacentor andersoni* and *D. variabilis* are the ticks most frequently involved in the transmission of this infection from small mammals, particularly rabbits, to humans. A number of tick species maintain the infection in the reservoir population.

The relapsing fevers form a group of diseases with a similar clinical pattern; they are caused by spirochetes of the genus *Borrelia*, all of which are transmitted by arthropod vectors. Lice transmit epidemic louse-borne relapsing fever, and soft ticks transmit endemic tick-borne relapsing fever.²⁹ The human relaps-

ing fevers are described as acute infections with toxemia and febrile periods that subside and recur over a period of weeks. Ticks of the genus *Ornithodoros* transmit tick-borne relapsing fevers. In the Western Hemisphere, *O. hermsi*, *O. turicata*, and *O. rudis* are the most important vectors. A close association between humans, vector ticks, and rodents infected with spirochetes, usually in a rural setting, is the typical condition necessary for human infections. In Africa, *O. moubata*, which feeds primarily on humans and lives in human dwellings, maintains transmission of relapsing fever from human to human.

Lyme arthritis and erythema chronicum migrans, or Lyme disease, as these conditions are known collectively, is caused by the spirochete *Borrelia burgdorferi*, which is transmitted by several ixodid ticks. *I. scapularis* (Fig. 39.7) is the primary vector in the eastern United States, while *I. pacificus* is the main vector on the West Coast, and *I. ricinus* is primarily responsible for transmission in Europe.³⁰⁻³⁴

The spirochete is commonly found in rodents, especially the white-footed mouse. In its immature stages, the tick vector feeds on this rodent reservoir host, and on deer in its adult stage. The range of the tick is limited by the range of the deer populations.



Figure 39.7. Adult female and nymph of *Ixodes scapularis*. Courtesy of A. Spielman and P. Rossignol.

Human infection with this spirochete usually results from a bite of the nymphal tick, though adult ticks are also capable of transmitting it. Before the tick has fed, the spirochete is found in its gut. After the tick has attached to a host and has begun feeding, the spirochetes disseminate throughout the hemocoel, invade the salivary glands, and infect the host. This process takes about a day, so prompt removal of the tick reduces the chance of infection.

Protozoal Diseases

Babesiosis, a protozoal disease, is seen in a variety of animals but rarely appears in humans. This disease, with its original focus of human cases on Nantucket Island and other islands off Cape Cod, Massachusetts, has spread to the U.S. mainland.¹⁴

Prevention and Control of Tick-Transmitted Infections

The best method of tick control is avoidance of areas where ticks are known to exist. Wide-scale chemical control of tick populations is impractical, although various compounds have been used. Tick control on dogs can be achieved with systemic compounds, topically applied chemicals, or available tick collars. Dusts have proved useful for preventing the introduction of brown or American dog ticks into homes. Permethrin, sprayed on clothing, appears to be most effective and may last at least a week or more.³⁵ DEET has been shown to have limited efficacy as a tick repellent and picaridin might be a reasonable alternative.^{35, 36}

Careful examinations for ticks are still necessary after traveling through infested areas. Because deer are one of the hosts of the tick vector, control of the over-abundant deer populations can be efficacious. Total elimination of a deer population on an isolated island (Mohegan Island, Maine, U.S.A.) eliminated the tick vector and stopped trans-

mission of the Lyme spirochete.³⁷ Significant reduction of deer populations at other sites had a major effect on density of tick populations and the frequency of transmission.^{38, 39} In one study, feeding deer ivermectin-medicated food had a significant effect on a population of vector ticks.⁴⁰

Mites

Within the Order Acari, the term “mite” is applied to members of several large families of minute arthropods, most free-living but many existing as ecto- or endoparasites of vertebrates and invertebrates. Mites affect humans by causing dermatitis. They serve as vectors of a number of diseases and as a source of allergens that can lead to serious hypersensitivity reactions.

Mites, as described by Aristotle, were well known to ancient civilizations. Their func-

tion as ectoparasites was not recognized until about the year 1000 CE, when scabies was first recorded. Although scabies continued to be described in the early medical literature, and physicians and naturalists repeatedly noted the association of a mite with this skin condition, the causal relation was largely ignored by the medical profession. In 1834, the demonstration that a mite was indeed the cause of scabies took place when a Corsican medical student recovered mites from affected individuals.⁴¹

Human Itch Mite: *Sarcoptes scabiei*

Scabies is a human skin disease caused by the mite *Sarcoptes scabiei* (Figs. 39.1, 39.8). It is usually associated with crowded living conditions, and its outbreaks often accompany wars, famine, and human migrations. Currently, scabies has reached pandemic proportions.⁴¹

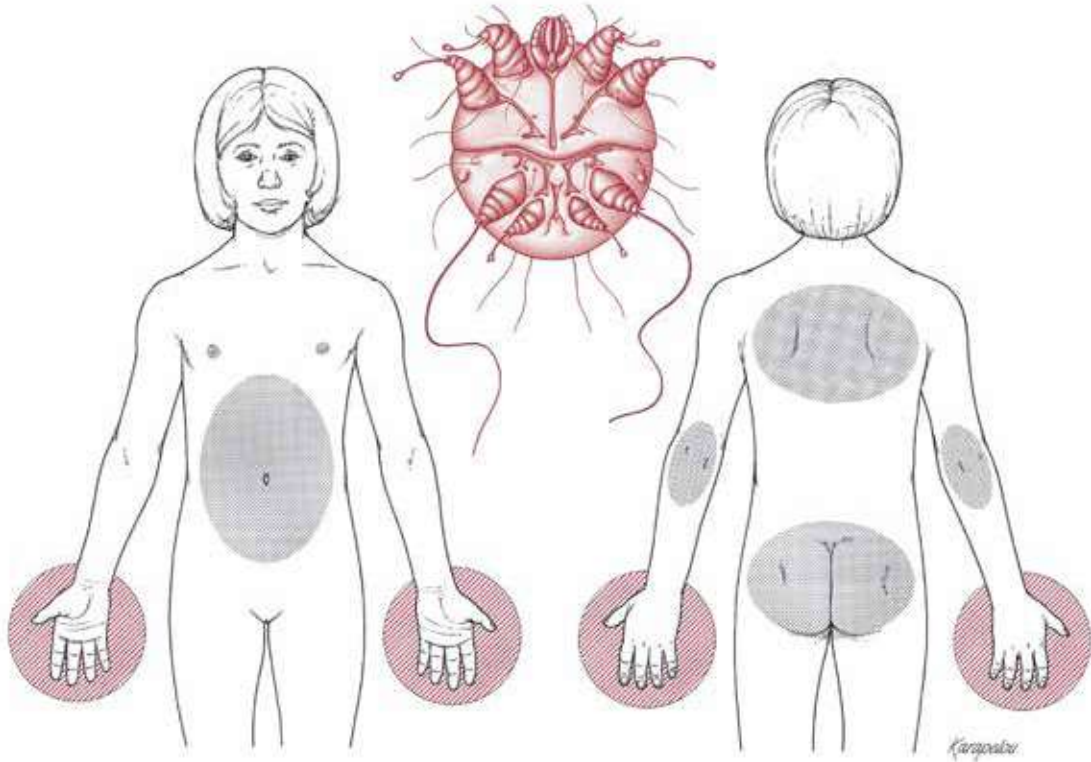


Figure 39.8. The itch mite, *Sarcoptes scabiei*. The stippled areas represent regions of the body where the rash is found. The mites themselves are found predominantly between the fingers and on the wrists (hatched areas).



Figure 39.9. Adult female itch mite, *Sarcoptes scabiei*.

The condition first presents as nocturnal itching, usually on the webbing and sides of the fingers, later spreading to the wrists, elbows, and the rest of the body. The buttocks, breasts of females, and genitalia of men are occasionally affected. Lesions appear as short, sinuous, slightly raised, cutaneous burrows.

Infections begin when fertile female mites (Fig. 39.9) are transferred from infected individuals by direct contact. The female mite



Figure 39.10. Scabies rash.

finds a suitable site, burrows into the skin, and tunnels through the upper layers of the epidermis, depositing fertile eggs. Six-legged larvae hatch from these eggs, leave the tunnel, and wander about the skin before re-invading it and starting new burrows. Once in place, the larvae eat, molt, and transform into eight-legged nymphs. Larvae destined to become females molt again into a second nymph stage. Those destined to become males molt directly to the adult form. After fertilization, young adult females begin construction of a new tunnel. The egg-to-egg life cycle may be as short as two weeks. A typical infection usually involves only 10–15 adult female mites.^{42–45}

With primary infections, itching and skin eruption are usually delayed for several weeks. As sensitization develops, the typical scabies rash appears on various parts of the body that do not necessarily correspond to the location of active adult female mites but represent a generalized response to the allergens (Fig. 39.10).

The face and scalp may be affected in infants and children, whereas adults seldom have lesions in these areas. A rare condition known as Norwegian, or crusted scabies may result from hyper-infection with thousands to millions of mites (Fig. 39.11). The consequence is a crusted dermatosis of the hands and feet



Figure 39.11. Patient with Norwegian scabies showing numerous lesions. Millions of mites may be present. Courtesy of Y. Mumcoughlu.



Figure 39.12. Large numbers of scabies mites and lesions on an immunosuppressed patient.

and often much of the body. This condition is characteristic of infected individuals who cannot take care of themselves and is often reported in custodial institutions such as mental hospitals.^{46, 47} It is highly contagious because of the large number of loosely attached, easily transferred mites present in the exfoliating skin. Crusted scabies has also been reported in individuals treated with immunosuppressive drugs (Fig. 39.12). Secondary infections of lesions, particularly as a result of scratching, are common; and post-streptococcal glomerulonephritis has been reported.⁴⁸

A diagnosis of scabies can be confirmed by picking up adult female mites at the ends of their burrows or by scraping the affected skin lightly covered with mineral oil. The scrapings are then examined under a microscope for immature or adult mites or for eggs. Skin biopsy reveals mites in tissue sections. The introduction of dermoscopy can aid in the diagnosis and allow for visualization of burrows as well as the visualization of mites as hanglider-like triangles.⁴⁹ The presence of infection in several members of a family is reasonable circumstantial evidence for a diagnosis of scabies in those not yet examined.

Lindane, the γ -isomer of benzene hexachloride, and permethrin are the most effective treatments available for treatment of scabies,

but lindane may have significant neurotoxicity. Lindane is available as a lotion or cream, which is applied once to the affected areas. Permethrin 5% cream is applied and left on for 8–14 hours. Benzyl benzoate in a 25% emulsion is an alternative drug, but must be applied to the whole body. Treatment of all members of a family may be necessary to prevent reinfection. Systemic treatment with ivermectin has proven particularly efficacious, but should be used with care.^{50, 51}

Animal Scabies

The itch and mange mites of various domestic animals (horses, pigs, dogs, cats, camels) can infest humans. These mites are often morphologically indistinguishable from the human parasites and are fully capable of penetrating human skin. The infection is usually self-limited because these mites do not form tunnels and cannot complete their life cycles. Humans may react with severe papular urticaria to these transitory infestations.

Chigger Mites

The chiggers, or redbugs, (Fig. 39.13) of the family Trombiculidae comprise an important group of annoying human ectoparasites. In



Figure 39.13. Larval stage of the common chigger *Trombicula alfreddugesi*. It is the larva, with three pairs of legs, that feeds on vertebrates. Photo by D. Scharf.

some geographic areas, they act as vectors of the organism causing scrub typhus (*Orientalis tsutsugamushi*).

In the United States, the chigger mites include three species of *Trombicula*. Among the chiggers, only the six-legged larvae feed on humans and other mammals, whereas the nymphs and adults usually feed on arthropods or arthropod eggs. Chigger larvae are usually picked up in brush frequented by rodents or other small mammals, which serve as normal hosts of the larvae. These mites tend to attach to skin where clothing is tight or restricted. The ankles, waistline, armpits, and perineal skin are common areas of infestation. Chiggers insert their capitula but do not burrow into the skin. The host reacts to the mouthparts and the injected saliva by forming a tube-like “stylosome” partially engulfing the feeding mites. The chigger does not feed on blood; rather, it ingests a mixture of partially digested cells and fluids formed within the stylosome. After feeding for several days, the engorged chigger withdraws and drops to the ground.^{52, 53}

The intense itching and discomfort associated with chigger “bites” often begins after the chiggers have withdrawn and departed. Irritation may be so severe as to cause fever and loss of sleep. Local anesthetics may be useful for relieving itching, and antibiotics may be needed to treat secondary bacterial skin infections.⁵⁴

In areas where chiggers are common, repellents containing DEET applied to skin and clothing can be effective. Scrubbing exposed or infested areas of the body with soap and water removes even well attached chiggers.

Scrub typhus (*Orientalis tsutsugamushi*) is a chigger-borne gram-negative coccobacillus zoonotic disease found in Southeast Asia, certain islands in the Indian and Pacific Oceans,

and Australia that is antigenically distinct from rickettsiae. The usual vectors are larvae of the chigger *Trombicula akamushi* and *T. deliensis*. Rodents are the normal reservoir hosts for this pathogen.⁵⁵

Follicle Mites

The ubiquitous follicle mite *Demodex folliculorum* are normal inhabitants of our sebaceous glands and hair follicles, particularly around the nose and eyelids. Follicle mites are minute (<0.4 mm long), atypically vermiform arthropods that seldom cause discomfort.⁵⁶⁻⁵⁸ In rare cases, the skin of the scalp becomes heavily infected. Follicle mites have been implicated as the cause of some forms of rosacea and blepharitis. Treatment consists of a topical application of ivermectin cream.⁵⁹ Closely related species of *Demodex*, which cause mange in dogs and other mammals, may cause a transitory burning reaction in individuals handling heavily infested animals.

Mites and Dermatitis

A number of species of mites, either parasitic for animals or free-living, occasionally infest humans and cause dermatitis. Mites associated with straw, flour, grain, dried fruits, vanilla, copra, and cheese can produce serious but transitory skin irritation in persons contracting large numbers. Bird and rodent mites may also cause serious annoyance when they attempt to feed on humans, but they do so only occasionally. Bird mites may be particularly bothersome if their normal avian hosts depart and they are forced to forage for food.

The tropical rat mite, *Ornithonyssus bacoti*, is a parasite of rodents that can attack humans and cause dermatitis.^{43, 60} The mite *Allodermanyssus sanguineus* is a common ectoparasite of mice, but readily feeds on humans. These mites have been shown to transmit rickettsial pox (*Rickettsia akari*), a mild exanthematous disease related to Rocky Mountain spotted

fever found in the eastern United States and Russia.⁵⁵

Allergies Caused by Mites

Certain mites of the genus *Dermatophagoides* have been incriminated as sources of antigens associated with allergies to house dust.⁴⁵ House dust mite allergens are a common cause of asthma attacks in sensitized individuals, particularly children. Desensitization has been successful.⁶¹⁻⁶⁶

Araneae (Spiders)

The spiders constitute a large, distinctive order of arachnids whose bodies are divided into two regions: cephalothorax and abdomen. Four pairs of walking legs, pedipalps, and chelicera (which house venom glands) all arise from the cephalothorax. Most, but not all, spiders produce venom in anterior venom glands that is capable of immobilizing prey. Although most species are unable to pierce human skin, several groups of spiders do occasionally bite humans. Spiders inject toxins that can cause severe systemic or tissue reactions. It is important to note that spider venom is used primarily for hunting rather than defense and that defensive bites occur as a last resort in most instances. The consequences of these bites may include transitory pain, necrotic lesions, systemic reactions, or even death.⁶⁷



Figure 39.14. Black widow spider. Ventral view.

Tarantulas

The name tarantula is loosely applied to a number of large, hairy spiders, some of which belong to the family *Theraphosidae*. These spiders are common in tropical and subtropical regions. Tarantulas are kept as house pets by many individuals and are regularly bred in captivity. Despite their reputation, few tarantulas bite humans and bites are both rare and not particularly troublesome. Tarantulas do have urticating hairs that may lead to skin irritation in some individuals. Bites inflict a painful wound, but the symptoms are not long lasting, and no fatalities have been reported.

Black Widow Spiders

Spiders of the genus *Latrodectus* are found throughout the world, primarily in warm climates. At least six species impact human health. They inflict painful and rarely fatal wounds (mostly in children).⁶⁸⁻⁷⁰ *Latrodectus mactans*, the black widow, hourglass, or shoe-button spider, (Fig. 39.14) is widespread throughout the United States and southern Canada. Related species are found throughout the temperate and tropical regions of all continents. The adult female black widow spider is usually black with a characteristic crimson hourglass marking on the underside of its globose abdomen. The coloration (various shades of black, gray, or brown) and the shape of the hourglass may vary. The typical mature female is about 40 mm long with its legs extended. Black widow spiders are normally reclusive in behavior, but females bite if disturbed and are particularly aggressive when they are gravid or defending their egg cases. These spiders frequent wood and brush piles, old wooden buildings, cellars, hollow logs, and vacant rodent burrows. The pit privy is a preferred site for webs, and a significant number of human spider bites have taken place in these locations.

Bites of the black widow spider may be initially painless, sometimes appearing only as



Figure 39.15. *Loxocles reclusa*, the brown recluse spider. Dorsal view. Note "fiddle" pattern.

two small red puncture marks at the site. Subsequently, pain at the site increases, spreads, reaching a maximum within 1–3 hours, and later subsides. Generalized muscle pain, abdominal rigidity, tightness in the chest, difficulty with breathing and speaking, nausea, and sweating may occur within an hour of the bite. Most symptoms pass after 2–3 days without treatment. In severe cases, paralysis and coma may precede cardiac or respiratory failure. The toxin has been identified as a low-molecular-weight protein. Its mode of action involves the inhibition of fusion of neurotransmitter vesicles with membranes leading to depolarization of synapses.^{71–74} Treatment usually consists of measures designed to relieve pain and reduce muscle spasms. Anti-venom is available in locales where bites are common.^{75,76} Control of black widow spiders with the use of insecticides such as malathion, particularly in privies, is effective.

Necrotic Arachnidism

Five species of the genus *Loxocles* in the New World attack when they are disturbed. Their bites may produce severe tissue reactions. *Loxocles reclusa* (Fig. 39.15) is found

in the southern and central United States; *L. unicolor* and *L. arizonica* are found in the western states; and *L. laeta* and *L. intermedia* are seen in South America. These spiders are of medium size, are yellow to brown in color, and have a body length of 10–15 mm.⁷⁷

Loxocles reclusa, the brown recluse spider of the United States, is a non-aggressive arachnid found outdoors in woodpiles and debris in warm climates, and in basements or storage areas in cooler regions. Humans are typically bitten only when they disturb them (e.g., when entering a sleeping bag, or putting on shoes or clothing).⁵⁴ Bites from this group of spiders are vastly over diagnosed relative to the number of actual verified bites.

The South American brown spiders, *L. laeta* and *L. intermedia*, are common domestic species found in closets, corners of rooms, or behind pictures. Humans are often bitten while sleeping or dressing, but usually only when the spider is threatened or disturbed. *L. laeta* has been introduced into the United States on at least one occasion.⁷⁸

The bite of *Loxocles* tends to be initially painless. Several hours later, itching, swelling, and tenderness may develop in the area of the bite. The wound site may turn violaceous and then black and dry. In other cases, a



Figure 39.16. Spider bite - *Loxocles* spp. initial bite on the tip of the thumb.



Figure 39.17. Scorpion.

blister may form over the bite. Necrosis may begin within 3–4 days, and tissue destruction may be extensive (Fig. 39.16). Healing may take eight weeks or longer. Some of the more serious lesions require surgery and skin grafts. The venom of *Loxocles* appears to work by inactivating hemolytic components of complement.^{79–85} An antiserum for treatment of *Loxocles* bites is being evaluated in Brazil. *Loxocles* spiders may be controlled in dwellings with insecticide compounds containing γ -benzene hexachloride or malathion.

Chiracanthium mildei is the most common spider found in houses in the eastern United States; usually in bathrooms, kitchens, and bedrooms. It attacks when disturbed, and its bite can cause a mild necrotizing skin lesion.^{86–93} Spiders of the genera *Phoneutria* in Brazil and Chile are capable of inflicting severe bites, sometimes with fatal results.

The spider fauna of Australia is particularly

robust and contains many dangerous species, but only the male *Atrax* spider can inflict a lethal bite in humans. The venom is neurotoxic and causes nausea, vomiting, abdominal pain, diarrhea, profuse sweating, salivation, and lacrimation. There may also be severe hypertension and cardiac arrest.

Scorpiones (Scorpions)

The scorpions belong to the order Scorpiones of the class Arachnida, with all members generally similar in appearance (Fig. 39.17). The typical scorpion is an elongate arthropod with stout, crab-like claws (pedipalps), four pairs of walking legs, with a distinctly-segmented abdomen and metasoma ending in a hooked stinger.⁹⁴

Scorpions are reclusive, nocturnal animals that feed primarily on other arthropods and sometimes on small rodents. While feeding, the scorpion holds its prey with its pedipalps and repeatedly stings its victim with over-the-back thrusts of its stinger. When the scorpion is disturbed, it uses the stinger for defense, which is the manner in which humans are stung. Most species of scorpions are unable either to penetrate human skin or inject sufficient toxin to cause damage. The few species that do sting humans are capable of inflicting a painful wound, precipitating a severe reaction and sometimes causing death. These species present a significant hazard to public health in many tropical and sub-tropical regions.⁹⁵

Scorpions produce two types of venom: hemolytic and neurotoxic. The first induces local reactions characterized by a burning sensation, swelling, and necrosis at the wound site. The second produces intense pain at the site of the sting and causes chills, cold perspiration, thirst, excessive salivation, and vomiting. Other systemic symptoms may include generalized numbness, difficulty with

speech and swallowing, paralysis, convulsions, tachycardia, and myocarditis. Death may result from respiratory paralysis, often within two hours of the sting.

Children under five years of age are particularly susceptible to the adverse effects of scorpion stings, and case fatality rates of 5% in Mexico, 25% in Trinidad, and 60% in the Sudan have been reported. Multiple stings, stings around the head, and stings of debilitated individuals are also particularly serious. Scorpion stings are not uncommon in the western United States.⁹⁶

Since the venom is injected into the subcutaneous tissues, initial treatment of scorpion stings should be designed to delay absorption of the toxin into the lymphatic vessels. The affected limb should be immobilized and the sting area cleaned with soap and water. Ice should be applied to the wound site and the patient kept calm. Specific scorpion antisera are available in areas where stings are common.⁹⁷⁻¹⁰⁰

Programs to reduce scorpion populations with wide-scale or focal application of persistent chemical pesticides have met with limited success. Elimination of rubbish piles around dwellings can reduce favored hiding and breeding places of scorpions.

References

1. Bowman, A. S.; Nuttall, P. A., Ticks: Biology, Disease and Control. Cambridge University Press. **2009**.
2. Sonenshine, D. E., Biology of Ticks Volume 2. **2013**.
3. Smith, T.; Kilbourne, F. L., Investigations into the Nature, Causation, and Prevention of Texas or Southern Cattle Fever. **1893**.
4. Marchoux, E.; Salimbeni, A., La spirillose des poules. *Ann Inst Pasteur Paris* **1903**, *17*, 569-580.
5. Ross, P. H.; Milne, A. D., "Tick Fever." *British medical journal* **1904**, *2* (2291), 1453-4.
6. Bratton, R. L.; Corey, R., Tick-borne disease. *Am Fam Physician* **2005**, *71* (12), 2323-30.
7. de la Fuente, J., et al., Overview: Ticks as vectors of pathogens that cause disease in humans and animals. *Front Biosci* **2008**, *13*, 6938-46.
8. Meagher, K. E.; Decker, C. F., Other tick-borne illnesses: tularemia, Colorado tick fever, tick paralysis. *Dis Mon* **2012**, *58* (6), 370-6.
9. Pujalte, G. G.; Chua, J. V., Tick-borne infections in the United States. *Prim Care* **2013**, *40* (3), 619-35.
10. Buckingham, S. C., Tick-borne diseases of the USA: Ten things clinicians should know. *J Infect* **2015**, *71 Suppl 1*, S88-96.
11. Anderson, J. F., The natural history of ticks. *Med Clin North Am* **2002**, *86* (2), 205-18.
12. Spielman, A., et al., Human babesiosis on Nantucket Island, USA: description of the vector, *Ixodes (Ixodes) dammini*, n. sp. (Acarina: Ixodidae). *Journal of medical entomology* **1979**, *15* (3), 218-34.
13. Colyar, M., Tick removal techniques. *Adv Nurse Pract* **2006**, *14* (5), 26-7.
14. Spielman, A., et al., Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annual review of entomology* **1985**, *30*, 439-60.
15. Oliver, J. H., Jr., et al., Conspecificity of the ticks *Ixodes scapularis* and *I. dammini* (Acari: Ixodidae). *J Med Entomol* **1993**, *30* (1), 54-63.
16. Burgdorfer, W., et al., The western black-legged tick, *Ixodes pacificus*: a vector of *Borrelia burgdorferi*. *The American journal of tropical medicine and hygiene* **1985**, *34* (5), 925-30.
17. Jupp, P. G., et al., Attempts to transmit hepatitis B virus to chimpanzees by arthropods. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde* **1991**, *79* (6), 320-2.

18. Sen, S. K., The mechanism of feeding in ticks. *Parasitology* **1935**, 27, 355-368.
19. Greenstein, P., Tick paralysis. *Med Clin North Am* **2002**, 86 (2), 441-6.
20. Pecina, C. A., Tick paralysis. *Semin Neurol* **2012**, 32 (5), 531-2.
21. Vedanarayanan, V. V., et al., Tick paralysis in children: electrophysiology and possibility of misdiagnosis. *Neurology* **2002**, 59 (7), 1088-90.
22. Diaz, J. H., A Comparative Meta-Analysis of Tick Paralysis in the United States and Australia. *Clin Toxicol (Phila)* **2015**, 53 (9), 874-83.
23. Felz, M. W., et al., A six-year-old girl with tick paralysis. *N Engl J Med* **2000**, 342 (2), 90-4.
24. Burgdorfer, W., Tick-borne diseases in the United States: Rocky Mountain spotted fever and Colorado tick fever. A review. *Acta tropica* **1977**, 34 (2), 103-26.
25. Burgdorfer, W.; J., A review of Rocky Mountain spotted fever (tick-borne typhus), its agent, and its tick vectors in the United States. *Entomol* **1979**, 12, 269-278.
26. McDade, J. F., et al., Ehrlichiosis, Q fever, typhus, rickettsialpox, and other rickettsioses. 1998; p 1599-1611.
27. Ijdo, J. W., et al., The emergence of another tick-borne infection in the 12-town area around Lyme, Connecticut: Human Granulocytic Ehrlichiosis. *Dis 181* **2000**, 4.
28. Diuk-Wasser, M. A., et al., Coinfection by *Ixodes* Tick-Borne Pathogens: Ecological, Epidemiological, and Clinical Consequences. *Trends Parasitol* **2016**, 32 (1), 30-42.
29. Burgdorfer, W., et al., The epidemiology of relapsing fevers. 1976; p 191-200.
30. Burgdorfer, W., et al., Lyme disease-a tick-borne spirochetosis? *Science (New York, N.Y.)* **1982**, 216 (4552), 1317-9.
31. Burgdorfer, W., et al., Erythema chronicum migrans--a tickborne spirochetosis. *Acta tropica* **1983**, 40 (1), 79-83.
32. Basler, E. M., et al., Natural distribution of *Ixodes dammini* spirochete. *Science* **1983**, 220, 321-322.
33. Lane, R. S., et al., Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. *Annual review of entomology* **1991**, 36, 587-609.
34. Steere, A. C., et al., The spirochetal etiology of Lyme disease. *The New England journal of medicine* **1983**, 308 (13), 733-40.
35. Pages, F., et al., Tick repellents for human use: prevention of tick bites and tick-borne diseases. *Vector Borne Zoonotic Dis* **2014**, 14 (2), 85-93.
36. Insect repellents. *Med Lett Drugs Ther* **2016**, 58 (1498), 83-5.
37. Kugeler, K. J., et al., Will Culling White-Tailed Deer Prevent Lyme Disease? *Zoonoses Public Health* **2016**, 63 (5), 337-45.
38. Kilpatrick, H. J., et al., The relationship between deer density, tick abundance, and human cases of Lyme disease in a residential community. *J Med Entomol* **2014**, 51 (4), 777-84.
39. Rand, P. W., et al., Abundance of *Ixodes scapularis* (Acari: Ixodidae) after the complete removal of deer from an isolated offshore island, endemic for Lyme Disease. *J Med Entomol* **2004**, 41 (4), 779-84.
40. Pound, J. M., et al., Systemic treatment of white-tailed deer with ivermectin-medicated bait to control free-living populations of lone star ticks (Acari: Ixodidae). *J Med Entomol* **1996**, 33 (3), 385-94.
41. Busvine, J. R., Insects, Hygiene, and History. *Athlone Press London* **1976**.
42. Mellanby, K., Transmission of Scabies. *Br Med J* **1941**, 2 (4211), 405-6.
43. Rosamilia, L. L., Scabies. *Semin Cutan Med Surg* **2014**, 33 (3), 106-9.
44. Walton, S. F., et al., Scabies: new future for a neglected disease. *Advances in parasitology* **2004**, 57, 309-76.
45. Romani, L., et al., Prevalence of scabies and impetigo worldwide: a systematic review. *Lancet Infect Dis* **2015**, 15 (8), 960-7.
46. Hatter, A. D., et al., Case report of individual with cutaneous immunodeficiency and novel 1p36 duplication. *Appl Clin Genet* **2016**, 9, 1-4.
47. Karthikeyan, K., Crusted scabies. *Indian J Dermatol Venereol Leprol* **2009**, 75 (4), 340-7.
48. Guldbakke, K. K.; Khachemoune, A., Crusted scabies: a clinical review. *J Drugs Dermatol* **2006**, 5 (3), 221-7.

49. Park, J. H., et al., The diagnostic accuracy of dermoscopy for scabies. *Ann Dermatol* **2012**, *24* (2), 194-9.
50. Goldust, M., et al., Treatment of scabies: Comparison of permethrin 5% versus ivermectin. *J Dermatol* **2012**, *39* (6), 545-7.
51. Romani, L., et al., Mass Drug Administration for Scabies Control in a Population with Endemic Disease. *N Engl J Med* **2015**, *373* (24), 2305-13.
52. Sasa, M., Biology of chiggers. *Annu Rev Entomol* **1961**, *6* 221-244.
53. Audy, J. R., Red Mites and Typhus. *Athlone Press London* **1968**.
54. Schulert, G. S.; Gigante, J., Summer penile syndrome: an acute hypersensitivity reaction. *J Emerg Med* **2014**, *46* (1), e21-2.
55. Fuller, H. S.; J., Studies of rickettsial pox: Life cycle of the mite vector *Allodermanyssus sanguineus*. *I Am* **1954**, *59*, 236-239.
56. Elston, C. A.; Elston, D. M., Demodex mites. *Clin Dermatol* **2014**, *32* (6), 739-43.
57. Chen, W.; Plewig, G., Human demodicosis: revisit and a proposed classification. *Br J Dermatol* **2014**, *170* (6), 1219-25.
58. Cheng, A. M., et al., Recent advances on ocular *Demodex* infestation. *Curr Opin Ophthalmol* **2015**, *26* (4), 295-300.
59. Ali, S. T., et al., The treatment of rosacea with topical ivermectin. *Drugs Today (Barc)* **2015**, *51* (4), 243-50.
60. Cafiero, M. A., et al., Dermatitis by Tropical Rat Mite, *Ornithonyssus bacoti* (Mesostigmata, Macronyssidae) in Italian city-dwellers: a diagnostic challenge. *J Eur Acad Dermatol Venereol* **2015**.
61. Arlien, L. G.; Morgan, M. S., Biology, ecology, and prevalence of dust mites. *Immunol Allergy Clin North Am* **2003**, *23*, 443-468.
62. Milian, E., et al., Allergy to house dust mites and asthma. **2004**, *23* 47-57.
63. Bessot, J. C.; Pauli, G., Mite allergens: an overview. *Eur Ann Allergy Clin Immunol* **2011**, *43* (5), 141-56.
64. Huss, K., et al., House dust mite and cockroach exposure are strong risk factors for positive allergy skin test responses in the Childhood Asthma Management Program. *J Allergy Clin Immunol* **2001**, *107* (1), 48-54.
65. Raulf, M., et al., Mites and other indoor allergens - from exposure to sensitization and treatment. *Allergo J Int* **2015**, *24* (3), 68-80.
66. Ken, K. M., et al., Dermatoses associated with mites other than *Sarcoptes*. *Semin Cutan Med Surg* **2014**, *33* (3), 110-5.
67. Juckett, G., Arthropod bites. *Am Fam Physician* **2013**, *88* (12), 841-7.
68. Vetter, R. S.; Isbister, G. K., Medical aspects of spider bites. *Annu Rev Entomol* **2008**, *53*, 409-29.
69. Isbister, G. K.; Fan, H. W., Spider bite. *Lancet* **2011**, *378* (9808), 2039-47.
70. Kang, J. K., et al., Spiders in dermatology. *Semin Cutan Med Surg* **2014**, *33* (3), 123-7.
71. Nicholson, G. M.; Graudins, A., Spiders of medical importance in the Asia-Pacific: atracotoxin, latrotoxin and related spider neurotoxins. *Clin Exp Pharmacol Physiol* **2002**, *29* (9), 785-94.
72. Maretic, Z., et al., Venomous Animals and Their Venoms. 1971; p 299-309.
73. Russell, F. E., Venom Poisoning. *Rational drug therapy* **1971**, *5* (8), 1-7.
74. Sudhof, T. C., alpha-Latrotoxin and its receptors: neurexins and CIRL/latrophilins. *Annu Rev Neurosci* **2001**, *24*, 933-62.
75. Monte, A. A., Black widow spider (*Latrodectus mactans*) antivenom in clinical practice. *Curr Pharm Biotechnol* **2012**, *13* (10), 1935-9.
76. Monte, A. A., et al., A US perspective of symptomatic *Latrodectus* spp. envenomation and treatment: a National Poison Data System review. *Ann Pharmacother* **2011**, *45* (12), 1491-8.
77. Furbee, R. B., et al., Brown recluse spider envenomation. *Clin Lab Med* **2006**, *26* (1), 211-26, ix-x.

78. Levi, H. W., et al., Biology and control of the South American brown spider *Loxosceles laeta* (Nicolet) in a North American focus. *Am Med Hyg* **1964**, *13*, 132-136.
79. Rhoads, J., Epidemiology of the brown recluse spider bite. *J Am Acad Nurse Pract* **2007**, *19* (2), 79-85.
80. Wendell, R. P., Brown recluse spiders: a review to help guide physicians in nonendemic areas. *South Med J* **2003**, *96* (5), 486-90.
81. Vetter, R. S.; Barger, D. K., An infestation of 2,055 brown recluse spiders (Araneae: Sicariidae) and no envenomations in a Kansas home: implications for bite diagnoses in nonendemic areas. *J Med Entomol* **2002**, *39* (6), 948-51.
82. Vetter, R., Identifying and misidentifying the brown recluse spider. *Dermatology online journal* **1999**, *5* (2), 7.
83. Swanson, D. L.; Vetter, R. S., Bites of brown recluse spiders and suspected necrotic arachnidism. *The New England journal of medicine* **2005**, *352* (7), 700-7.
84. Williams, S. T., et al., Severe intravascular hemolysis associated with brown recluse spider envenomation. A report of two cases and review of the literature. *American journal of clinical pathology* **1995**, *104* (4), 463-7.
85. Zeglin, D., Brown recluse spider bites. *Am J Nurs* **2005**, *105* (2), 64-8.
86. Spielman, A.; Levi, H. W., Probable envenomation by *Chiracanthium mildei*; a spider found in houses. *The American journal of tropical medicine and hygiene* **1970**, *19* (4), 729-32.
87. Nimorakiotakis, B.; Winkel, K. D., The funnel web and common spider bites. *Aust Fam Physician* **2004**, *33* (4), 244-51.
88. Isbister, G. K., et al., Antivenom treatment in arachnidism. *J Toxicol Clin Toxicol* **2003**, *41* (3), 291-300.
89. Isbister, G. K., et al., Funnel-web spider bite: a systematic review of recorded clinical cases. *Med J Aust* **2005**, *182* (8), 407-11.
90. Isbister, G. K.; Gray, M. R., Bites by Australian mygalomorph spiders (Araneae, Mygalomorphae), including funnel-web spiders (Atracinae) and mouse spiders (Actinopodidae: Missulena spp). *Toxicon* **2004**, *43* (2), 133-40.
91. Gaudins, A., et al., Red-back spider (*Latrodectus hasselti*) antivenom prevents the toxicity of widow spider venoms. *Ann Emerg Med* **2001**, *37* (2), 154-60.
92. White, J., Envenoming and antivenom use in Australia. *Toxicon : official journal of the International Society on Toxinology* **1998**, *36* (11), 1483-92.
93. Currie, B. J., Clinical toxicology: a tropical Australian perspective. *Therapeutic drug monitoring* **2000**, *22* (1), 73-8.
94. Keegan, H. L., Scorpions of medical importance. 1980.
95. Del Brutto, O. H., Neurological effects of venomous bites and stings: snakes, spiders, and scorpions. *Handb Clin Neurol* **2013**, *114*, 349-68.
96. LoVecchio, F.; McBride, C., Scorpion envenomations in young children in central Arizona. *J Toxicol Clin Toxicol* **2003**, *41* (7), 937-40.
97. Tuuri, R. E.; Reynolds, S., Scorpion envenomation and antivenom therapy. *Pediatr Emerg Care* **2011**, *27* (7), 667-72; quiz 673-5.
98. Bawaskar, H. S.; Bawaskar, P. H., Scorpion sting: update. *J Assoc Physicians India* **2012**, *60*, 46-55.
99. Bosnak, M., et al., Severe scorpion envenomation in children: Management in pediatric intensive care unit. *Hum Exp Toxicol* **2009**, *28* (11), 721-8.
100. Chippaux, J. P., Emerging options for the management of scorpion stings. *Drug Des Devel Ther* **2012**, *6*, 165-73.



Karl Theodor Ernst von Siebold, M.D. (1804–1885)

Siebold was a generalist, contributing to such diverse fields as invertebrate taxonomy, parasite life cycles (*Schistosoma haematobium* in collaboration with Theodor Bilharz; *Fasciola hepatica*, *Echinococcus granulosus*), and establishing the journal: *Zeitschrift für Wissenschaftliche Zoologie*.

40. Other Arthropods of Medical Importance

Butterflies and Moths: Order Lepidoptera

Several species of larvae or caterpillars of the order Lepidoptera are covered with hollow, sharply pointed hairs containing a toxin that may cause severe dermatitis. Contact occurs when an individual handles the caterpillars or inhales the “hairs,” which are blown about after the larva molts. In the northeastern United States, the gypsy moth, *Lymantria dispar*, has been responsible for defoliation of enormous areas of forest. The “population explosion” of the caterpillars of this species has led to periodic outbreaks of pruritic dermatitis, primarily among school children.¹ Similar outbreaks of dermatitis have been reported from the Southeastern and South Central U. S., attributed to the caterpillars of the puss moth *Megalopyge opercalis*.

In a Mexican outbreak, severe dermatitis was initially attributed to scabies, but eventually associated with contact with adult moths of the species *Hylesia alinda*. Populations of this normally rare species rapidly expanded after its natural predators were killed by a hurricane sweeping the island.² Lepidopteran pests include the Io moth, saddleback caterpillar, the puss caterpillar and many members of the family Limacodidae.

Individuals working with lepidopterans may become sensitized to the scales of adult moths or butterflies. Repeated exposure may produce severe bronchospasm and even asthma. There is an increasing awareness of the ubiquity and potentially serious consequences of exposure to a wide range of these larvae.³⁻⁵

Beetles: Order Coleoptera

Within the order Coleoptera, which comprises a large group of insects, only a few families contain members of medical importance. Certain scavenger beetles of the families Dermestidae, Silphidae, and Staphylinidae feed on feces and carrion and mechanically transmit pathogenic organisms. Adult beetles of the family Meloidae, the blister beetles, produce a vesicant (cantharidin) that may cause blistering or a severe burning sensation on contact with the skin or mucous membranes. Inadvertent ingestion of beetle larvae, a condition termed canthariasis, may produce transient gastrointestinal (GI) discomfort.

Many beetles, particularly those that feed on feces, act as intermediate hosts for helminthic parasites of humans and other animals. Members of the family Scarabaeidae are intermediate hosts of the spiny-headed worm *Macracanthorhynchus hirudinaceus*, a parasite of pigs, which rarely infects humans. The tapeworms *Hymenolepis nana* and *Hymenolepis diminuta* develop in grain beetles of the family Tenebrionidae.

Cockroaches: Order Blattodea

Blattodea is a large, diverse order of primitive, successful insects (over 4,000 species, worldwide) that includes the grasshoppers and crickets, as well as cockroaches. The cockroaches are included in a single family, the Blattidae, with several members closely associated with human habitations. All members of this group have chewing mouthparts.

The female cockroach encloses her eggs in a bean-shaped case called an ootheca. Some species retain the ootheca internally until the eggs hatch, others carry it externally for several weeks, and still others drop the ootheca soon after it is formed. After hatching, the young, wingless, feeding nymphs begin to

undergo staged development. Some species progress through as many as 13 nymph stages, each being wingless and somewhat larger than its predecessor, until the final molt produces the winged adult. With its series of wingless nymph stages, the cockroach is a classic example of an insect developing by incomplete metamorphosis.

Most cockroach species do not invade homes, confining themselves to outdoor habitats, although in the United States eleven species of cockroaches do invade human habitats. The most common is the German cockroach or croton bug, *Blattella germanica*, a small (<16 mm), light brown species. The American cockroach or palmetto bug *Periplaneta americana*, is, in fact, an African species now found worldwide. It is a large (30–40 mm) reddish-brown insect with long wings. It is found in and around homes, farms, restaurants, stores, and warehouses. Other species that may infest homes are the Oriental cockroach, *B. orientalis*, the Australian cockroach, *P. australasiae*, and the brown-banded cockroach *Supella longipalpa*.

Most of these species are cosmopolitan, having been distributed by ship traffic starting with the earliest voyages. In general, domestic species are omnivorous. They feed on a wide variety of nutrients including: paper; bookbindings; and human and animal feces. They serve as mechanical vectors of pathogens, carrying infectious agents from feces to food.⁶

The presence of cockroaches is usually associated with a breakdown of general sanitation. Exposed foods or poor packaging and storage, open garbage, darkness and moisture are all conducive to the development of large cockroach populations. Initial infestations may be introduced with foodstuffs or migration from adjoining dwellings. In apartment buildings, the insecticide treatment of one apartment may cause the migration of cockroaches to adjoining, untreated apartments.

Cockroach baits paired with sanitation, best control cockroach infestations. Although cockroaches are resistant to a number of insecticides in some areas, compounds for control are commercially available. Coupled with improved housekeeping, treatment with these agents can be sufficient, although heavy infestations require repeated treatments by professional exterminators.

Cockroaches, because of their close association with sewage and garbage, may serve as paratenic hosts for various pathogens.⁶ Long-term exposure to cockroaches or to their shed exoskeletons can induce asthma-like symptoms.^{7,8}

Centipedes: Class Chilopoda

The centipedes of the class Chilopoda are worm-like, segmented creatures with a distinct head and paired appendages on each of 15–100 or more segments. They have a pair of poisonous claws, or maxillipeds, on the first segment after the head, which are used for capturing prey. Most centipedes are predaceous insectivores, and humans are sometimes bitten accidentally. Centipede bites may be locally painful, causing transient swelling at the site of the bite. No long-term complications are usually associated with these bites.⁹

Crustacea

The crustacea include many species that serve as intermediate hosts of parasites of humans and other animals. These organisms are discussed in the other, relevant chapters.

Tongue Worms: Class Pentastomida

The pentastomids, or tongue worms, of the class Pentastomida are a small group of parasites of uncertain origin and affinity. Because their larvae superficially resemble the larvae of mites, they have been included among the

Arthropoda, but they probably evolved early from annelid or arthropod ancestral stocks. They were first noted in the nasal cavities of dogs and horses during the eighteenth century and were later described in human autopsy material as insect larvae.

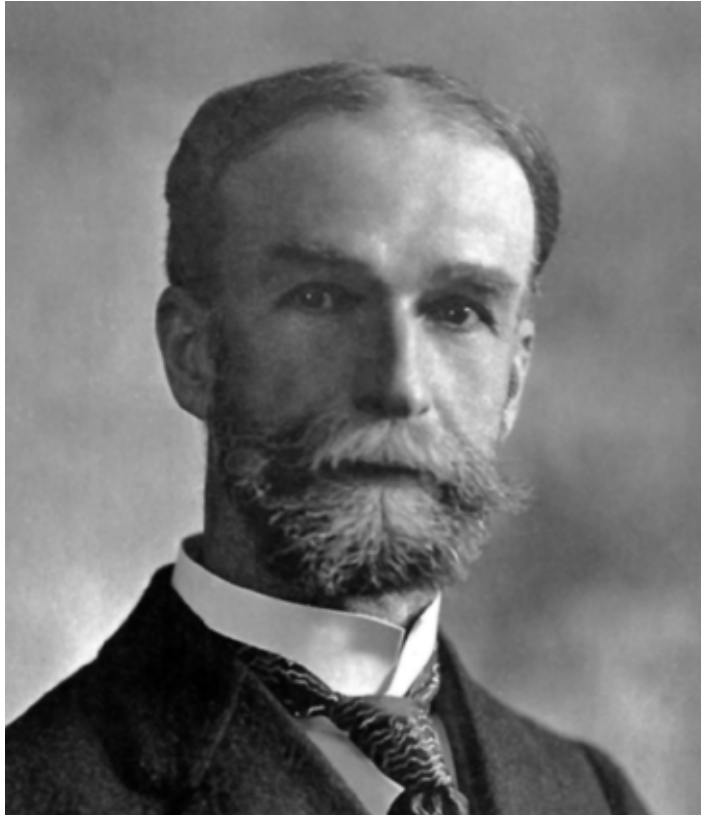
The adult tongue worms are blood sucking, endoparasitic, legless vermiform inhabitants of the respiratory system of reptiles, birds, and mammals. Eggs fertilized within the host emerge through the respiratory tract. After being eaten by an intermediate host, they

hatch in the gut, yielding a migratory larva that pierces the stomach wall and encysts in host tissue. When the intermediate host is eaten by the definitive host, the larvae mature.¹⁰

In humans, encysted larvae have been found in the lungs, liver, intestine, spleen, and other internal organs.^{11, 12} There may be rare cases of symptomatic human disease from infection with tongue worms, but most cases are usually identified at autopsy without prior attributable symptoms.¹¹⁻¹⁵

References

1. Allen, V. T., et al., Gypsy moth caterpillar dermatitis--revisited. *J Am Acad Dermatol* **1991**, 24 (6 Pt 1), 979-81.
2. Fernandez, G., et al., Epidemic dermatitis due to contact with a moth in Cozumel, Mexico. *The American journal of tropical medicine and hygiene* **1992**, 46 (5), 560-3.
3. Diaz, J. H., The evolving global epidemiology, syndromic classification, management, and prevention of caterpillar envenoming. *The American journal of tropical medicine and hygiene* **2005**, 72 (3), 347-57.
4. Hossler, E. W., Caterpillars and moths. *Dermatol Ther* **2009**, 22 (4), 353-66.
5. Hossler, E. W., Caterpillars and moths: Part II. Dermatologic manifestations of encounters with Lepidoptera. *J Am Acad Dermatol* **2010**, 62 (1), 13-28; quiz 29-30.
6. Graczyk, T. K., et al., Mechanical transmission of human protozoan parasites by insects. *Clin Microbiol Rev* **2005**, 18 (1), 128-32.
7. Gao, P., Sensitization to cockroach allergen: immune regulation and genetic determinants. *Clin Dev Immunol* **2012**, 2012, 563760.
8. Pomes, A.; Arruda, L. K., Investigating cockroach allergens: aiming to improve diagnosis and treatment of cockroach allergic patients. *Methods* **2014**, 66 (1), 75-85.
9. Sanaei-Zadeh, H., Centipede bite. *Eur Rev Med Pharmacol Sci* **2014**, 18 (7), 1106-7.
10. Drabick, J. J., Pentastomiasis. *Rev Infect Dis* **1987**, 9, 1087-1094.
11. Tappe, D.; Buttner, D. W., Diagnosis of human visceral pentastomiasis. *PLoS Negl Trop Dis* **2009**, 3 (2), e320.
12. Tappe, D., et al., Imported Armillifer pentastomiasis: report of a symptomatic infection in The Netherlands and mini-review. *Travel Med Infect Dis* **2014**, 12 (2), 129-33.
13. Latif, B., et al., Human pentastomiasis caused by *Armillifer moniliformis* in Malaysian Borneo. *Am J Trop Med Hyg* **2011**, 85 (5), 878-81.
14. Sulyok, M., et al., Ocular pentastomiasis in the Democratic Republic of the Congo. *PLoS Negl Trop Dis* **2014**, 8 (7), e3041.
15. Tappe, D., et al., Molecular Diagnosis of Abdominal Armillifer grandis Pentastomiasis in the Democratic Republic of Congo. *J Clin Microbiol* **2015**, 53 (7), 2362-4.



Theobald Smith, M.D. (1859–1934)

Smith and Fredrick L. Kilbourne co-discovered the cause of Texas cattle fever, *Babesia bigemina*, a protozoan parasite related to malaria. They also proved that the Lone Star Tick (*Amblyomma americanum*) transmitted it from cow to cow. This marked the first time that an arthropod was identified as a vector for an infectious disease. This seminal finding opened the door for a flood of other similar discoveries regarding the role that arthropods play in the spread of infectious diseases.

Appendix A: Procedures for Collecting Clinical Specimens for Diagnosing Protozoan and Helminthic Parasites

There is no substitute for a well-trained laboratory diagnostic technician, even with the advent of sensitive and specific serological and molecular diagnostic methods, such as ELISA, PCR, and NAAT. But even the best-trained personnel cannot make up for an improper sample delivered to the laboratory in the expectation of securing the diagnosis. Stool, blood, urine and tissue samples must be treated as the most important link between the patient and the correct diagnosis of their parasitic illness. The following advice outlines standard procedure for insuring that the diagnostic laboratory receives the right amount and type of patient specimen.

Stool Specimens

Proper collection and delivery of stool specimens is a critical aspect of any diagnostic procedure relying on stool examination. The clinician can control the quality of this aspect, and in doing so, will insure both the reliability and accuracy of any test they recommend, regardless of whether that test is carried out in-house or at a regional diagnostic facility.

1. Fresh, unpreserved feces should be obtained and transported to the laboratory immediately. Fresh specimens are preferred for examinations for trophozoites, and are required when tests for *Strongyloides stercoralis* larvae are to be performed.
2. Unpreserved feces should be examined within one hour after passage, especially if the stool is loose or watery, and might contain trophozoites of pathogenic amoebae. Examination of formed stool may be delayed for a short time, but must be completed on the day on

which the specimen is received in the laboratory. If prompt examination or proper fixation cannot be carried out, formed specimens may be refrigerated for 1-2 days.

3. If specimens are delayed in reaching the laboratory, or if they cannot be examined promptly (such as those received at night, on weekends, or when no parasitologist is available), portions should be preserved in fixatives such as 8% aqueous formalin or formol-saline, or with polyvinyl alcohol (PVA). Formalin preserves cysts, eggs and larvae for wet-mount examination or for concentration tests. PVA-fixative preserves trophozoites, cysts, and eggs for permanent staining. A ratio of one part feces to three parts of fixative is recommended. The specimen may be placed in fixatives in the laboratory, or the patient may be provided with fixatives and instructions for collection and preservation of their own specimens.

Stool Examination

Stool specimens may be successfully examined by any one of the three methods listed below. The advantages and limitations of each technique must be recognized.

1. Saline mounts are of value primarily for demonstrating the characteristic motility of amoebae and flagellates. In addition, seeing red cells inside a trophozoite of an amoeba is indicative of infection with *Entamoeba histolytica*. These organisms may be found in fresh stools, or occasionally in bloody mucus adhering to the surface of formed stools. Material should be obtained from several parts of the specimen. An iodine stain (a drop of 1% iodine in 2% potassium iodide) mixed with a stool suspen-

sion in saline solution facilitates identification of protozoan cysts, but it kills and distorts trophozoites.

2. Concentration techniques, useful for detecting small numbers of cysts and helminth eggs, may be used on unpreserved stool specimens, those preserved in aqueous formalin or formol-saline, or on PVA-fixed material.
3. Stained, thin smears of feces should be made, if possible, on all specimens obtained fresh or fixed in PVA. If properly prepared, they comprise the single most productive stool examination for protozoa. Smears may be stained with Wheatly-Gomori's Trichrome solution or with iron-hematoxylin (see Appendix B for colors of each). Any outstanding examples of positive specimens should be retained in a permanent file and used for future reference.

Number of Specimens Examined and Appropriate Intervals

1. To detect amoebae, a minimum of three specimens should be examined; if these samples (obtained preferably at intervals of 2-3 days) are negative and amoebic infection remains a diagnostic consideration, additional specimens should be examined.
2. With suspected giardiasis, NAAT and antigen capture ELISA are the currently recommended tests. When employing light microscopy, three specimens should initially be examined. If they are negative, additional specimens should be obtained at weekly intervals for three weeks.
3. A single concentrate from one day's worth of stool is frequently sufficient to

detect intestinal helminthic infections of clinical importance. With very light infections due to *Schistosoma* spp. few or no eggs may be found in the feces or urine. *Strongyloides stercoralis* may also require concentrating the specimen for diagnosis, but this method is not always reliable; various fecal culture methods may also be used.

4. Examination after treatment, under most circumstances, should be delayed until one month after completion of therapy (three months after treatment for schistosomiasis or tapeworms).

Examination of Blood

1. Smears for malaria should consist of both thick and thin films. It is important that all involved laboratory personnel be aware of the technique for making thick films as they are useless if improperly made. Smears should be stained with Giemsa solution and a minimum of 100 contiguous microscopic fields examined before a specimen is reported as "negative." If the first specimen is negative, additional thick and thin films should be taken every six hours for the first 24 hours after admission.
2. When examining for filarial infection, the possibility of diurnal or nocturnal periodicity of microfilariae in the peripheral blood must be taken into account, and specimens should be taken every six hours for the first 24 hours after admission, as with malaria. Thick smears or blood concentration methods are most likely to demonstrate infection. Smears should be done in conjunction with the Knott's test (see Appendix B).

Serologic Methods

A variety of immunodiagnostic methods may serve as useful adjuncts to the clinical diagnosis of parasitic infections. In some cases, serologic methods may be the only laboratory recourse for making a diagnosis. Certain serologic tests provide a high degree of diagnostic accuracy; however, mixed infections, cross reacting antigens by related and unrelated parasites, and other diseases or physiologic conditions may interfere with the diagnostic accuracy of a given test.

Western blot analysis and ELISA will undoubtedly continue to offer the clinician sensitive, reliable methods for diagnosing parasitic infections. Positive tests revealing the presence of specific antibodies are indirect evidence of infection, no matter how good the method. Tests employing antibody capture techniques, in which monoclonal antibodies are used to select for a single class of immunoglobulin increases the likelihood of a true positive result.

Most serum specimens may be shipped frozen or preserved with thimerosal to a final concentration of 1:10,000 to a state public health laboratory for forwarding to the Centers for Disease Control (CDC) and Prevention in Atlanta, Georgia. The vial, containing at least 2 ml of serum, should indicate the preservative used.

Nucleic Acid Amplification Tests (NAAT) and Polymerase chain reaction (PCR)

The advent of amplifying parasite DNA in stool, blood, and tissue samples has widened the range of parasite detection methods. Reliable NAAT tests have been developed for malaria, most species that cause leishmaniasis, toxoplasmosis, giardiasis, amoebiasis (*Entamoeba histolytica* and *E. dispar*), trypanosomiasis, and many others. These tests have already become the primary diagnostic tests in many clinical centers.



Charles Wardell Stiles, Ph.D. (1867–1941)

By 1900, the American South had not yet recovered from the economic impacts of the American Civil War. John D. Rockefeller appointed a special commission to look into the matter. The commission failed to identify a specific cause for the high rate of unemployment but did note that many people appeared to be suffering from anemia. In 1909, Rockefeller re-organized the commission, now known as the Rockefeller Sanitary Commission, and appointed Stiles as its head.

Stiles hypothesized that the anemia might be due to an infection similar to the one Dubini had described for tunnel workers in Northern Italy some years earlier. The commission's findings confirmed Stiles's suspicion. Hookworm infection compounded with an iron-poor diet were responsible for inducing the anemia. Stiles named the worm *Uncinaria americanus* (later re-named *Necator americanus*, the American killer). He also founded The Rockefeller Sanitary Commission for the Eradication of Hookworm which recommended several basic public health measures aimed at preventing the further spread of the disease: the installation of latrines, the use of tetrachlorethylene as an effective treatment for severe infections, and public health educational programs. Over the next ten years, the incidence and prevalence of hookworm infection sharply declined. Sanitation also lowered the incidence and prevalence rates of all fecally transmitted diseases, further improving the health of all and enabling many to do a full day's work.

Appendix B: Laboratory Diagnostic Methods

In recent years, a battery of cutting-edge diagnostic modalities have emerged, making the identification of many infectious diseases straightforward, without requiring any more skill than being able to read the instructions and to execute them. Microscopy training is not needed in these instances. Each of the preceding chapters attests to this fact, with a robust sampling of modern serological and molecular diagnostic strategies.

The vast majority of parasitology laboratories in hospitals and outpatient health clinics throughout the world continue to rely on more traditional approaches for the diagnosis of eukaryotic parasites. In these instances, microscopy remains the gold standard for pathogen identification. This chapter serves as a standard reference for these time-honored laboratory procedures.

Part I deals with unpreserved specimens and Part II with preserved specimens. There is no single method that efficiently renders all stages of all parasites available for microscopic identification; several tests must often be performed to obtain optimal results.

Unpreserved Stool Specimens

Ideally, stool specimens should be less than one hour old when first examined, although this may not always be possible. Stools that are up to 24 hours old may still be useful for recovering protozoan cysts, larvae and eggs of helminths, but trophozoites rarely survive that long. A confounding factor when examining specimens left at room temperature for more than 24 hours is that some parasites can grow and develop. Refrigeration helps prevent this problem. Stools should not be frozen, as that would alter the morphology of the organisms examined.

Because of day-to-day variability in the quantity of various stages of parasite shed by an infected individual, parasites may not be present in a single specimen, particularly when the infection is light. A total of three specimens collected on consecutive days are suggested when attempting to detect most enteric infections by visual microscopy. Some parasites (e.g., the schistosomes and *Giardia lamblia*) often require more specimens for detection or the use of more sensitive diagnostic modalities.

Barium or mineral oil interferes with identification of parasites. Patients should not be subjected to radiographic studies involving barium or given laxatives containing mineral oil until the stool specimens have been obtained.

Direct Examination

Gross examination:

1. Observe and record the appearance of the entire specimen, noting the color, consistency, and odor.
2. Examine the specimen for the presence of living parasites.
3. Perform a microscopic examination.
4. Examine a direct smear of the material.

The direct examination is effective for diagnosing living parasites (e.g., *Entamoeba histolytica*, *Giardia lamblia*, *Strongyloides stercoralis*), and should be performed on loose, diarrheic, or purged stool. When motile amoebae are found on a direct smear, a stained preparation should also be examined for the definitive diagnosis. If the amoeba contains erythrocytes within the cytoplasm, it is indicative of infection caused by *E. histolytica*.

If the specimen appears negative, as may occur with light infections, the sample should be concentrated.

1. Dip a wooden applicator stick into the specimen to coat the tip of it with stool.

2. Smear the stool onto a clear glass microscope slide on which a drop of normal saline solution has been placed and overlay with a coverslip. Smears must be thin enough to facilitate microscopic examination.

Staining the Direct smear

The Wheatly-Gomori trichrome reagent stains the protozoan nuclei red to dark blue, the cytoplasm a lighter blue, and the background material green. Trophozoites and cysts tend to shrink away from the background material and are therefore relatively easy to locate.

The Wheatly-Gomori trichrome reagent consists of:

- 6.0 g chromotrope 2R
- 0.5 g aniline blue CI42755
- 0.25 g dodecatungstophosphoric acid AR in 3 ml glacial acetic acid

Wheatly-Gomori trichrome stain is applied to a thin smear of stool on a coverslip, and the coverslip is immersed sequentially in the solutions enumerated below for the prescribed lengths of time.

Solution	Time
Schaudinn's fixative	5 minutes at 50+°C, or 1 hour at room temperature
Ethanol-iodine 70%	1 minute
Ethanol 70%	1 minute
Ethanol 70%	1 minute
Trichrome stain	2–8 minutes
Ethanol 90%	10–20 seconds (acidified)

To remove excess stain, briefly dip the coverslip in destaining solution once or twice. Rinse in 90% ethanol to stop the process. Thin smears destain quickly; thicker ones may require three or four dips to obtain optimal differentiation. The process is as follows.

Solution	Time
Ethanol 95% or 100%	Two rinses
Ethanol 100%	1 minute
Xylol	1 minute

Mount the coverslip in an appropriate mounting medium and examine under a microscope.

Concentration Methods

Sedimentation by Centrifugation:

Formaldehyde-Ethyl Acetate Method

Sedimentation by concentration and exposure to formaldehyde-ethyl acetate concentrates cysts and eggs of parasites, but debris and ether-soluble materials localize in the formaldehyde-ether interface or the ether layer in the top of the tube. This process destroys trophozoites, as they disintegrate in ethyl acetate.

1. Mix stool 1:10 with H₂O.
2. Strain through a single layer of gauze into a 15-ml centrifuge tube.
3. Centrifuge the strained stool (1 minute at 2000 rpm) and discard the supernatant.
4. Wash the sediment once with H₂O.
5. Repeat steps 3 and 4.
6. Discard the supernatant and save the sediment.
7. Add 10 ml of 7.5% formaldehyde to the sediment.
8. Let stand 10–30 minutes.
9. Add approximately 3 ml of ethyl acetate, plug the tubes with stoppers, and agitate the mixture vigorously.
10. Remove the stoppers and centrifuge the tubes at 1500 rpm for 1 minute.
11. Gently loosen the debris from the tube wall with an applicator stick, being careful not to disturb the pellet.
12. Discard the supernatant.
13. Examine the sediment microscopically.
14. Add a drop of 70% ethanol-iodine solution (Lugol's solution) and exam-

ine again if internal structures of cysts are not recognized on first examination.

Sedimentation by Gravity:

The water sedimentation test is used primarily for the concentration and recovery of *Schistosoma mansoni* and *Schistosoma japonicum* eggs, and it is effective for determining their viability. An entire day's worth of stool should be examined in a single test because schistosome eggs are shed sporadically.

1. Emulsify the entire stool sample in H₂O.
2. Strain the specimen through a single layer of gauze into conical sedimentation flasks.
3. Allow the sediment to settle (approximately 20 minutes) and discard the supernatant.
4. Resuspend the sediment in H₂O.
5. Repeat steps 3 and 4 until the supernatant is clear.
6. Discard final supernatant and save the sediment.
7. Examine the entire sediment microscopically.

The entire water sedimentation procedure should be done within two hours of starting, since prolonged exposure of schistosome eggs to water stimulates them to hatch. If hatching occurs, the empty shells remain in the sediment and the ciliated miracidia can be seen moving about rapidly.

Baerman Sedimentation Method

This method is extremely useful for concentrating and recovering larvae of *Strongyloides stercoralis*. The test requires a funnel with a piece of rubber tubing attached to it. An adjustable clamp is applied across the tubing, and the entire apparatus is suspended from a ring stand in a 37°C incubator. Larvae concentrate in the sediment that accumulates in the base of the rubber tube connected to the funnel, and the fluid containing them is

expressed into a test tube for microscopic identification

1. Emulsify the entire stool sample in H₂O.
2. Strain the specimen through a single layer of gauze into conical sedimentation flasks.
3. Allow the sediment to settle (approximately 20 minutes) and discard the supernatant.
4. Resuspend the sediment in H₂O.
5. Repeat steps 3 and 4 until the supernatant is clear.
6. Discard final supernatant and save the sediment.
7. Examine the entire sediment microscopically.

Floatation by Centrifugation:

Floatation methods concentrate various stages of parasites by taking advantage of their specific gravity. The unwanted debris sediments to the bottom of the tube during centrifugation, but the diagnostic forms float to the surface. Cysts and most eggs can be recovered in large quantities by this method, but trophozoites, operculated eggs, and schistosome eggs are either destroyed, or sediment to the bottom of the tube.

Zinc Sulfate Floatation Method

1. Mix 1 part stool in 15 ml H₂O in a 15-ml centrifuge tube.
2. Centrifuge for 1 minute at 2500 rpm; decant the supernatant.
3. Add zinc sulfate solution (specific gravity 1.18) until the tube is half full and resuspend the sediment with a wooden applicator stick.
4. Fill the tube to the top with more zinc sulfate solution.
5. Centrifuge the suspension for 1 minute at 2500 rpm. Do not apply the brake to the centrifuge or jar the tube, as either maneuver causes any eggs or cysts accumulated at the liquid-surface

interface to sink.

6. Using a bacteriologic loop, remove two aliquots from the surface and place them on a clean glass slide.
7. Examine microscopically. A small drop of Lugol's iodine solution can be added to provide contrast.

Sugar (Sheather's) Floatation Method

The recovery of *Cryptosporidium parvum* oocysts is facilitated by this method.

1. Filter stool through three pieces of cheesecloth.
2. Place 2 ml of stool filtrate in a conical tube.
3. Fill the tube to the top with sucrose solution.
4. Place a coverslip on top of the tube.
5. Centrifuge at 1000 rpm for 5 minutes. If the stool sample is watery, no centrifugation is necessary. Let the coverslip rest on the top of sucrose solution for 20 minutes.
6. Examine the coverslip microscopically at 400 X. The focal plane is important, because the oocysts are located on the inner surface of the coverslip, rather than on the slide itself. The oocysts appear slightly pink in color without the addition of any stain. They are ovoid to spherical in shape, range in size from 5 to 6 μm in diameter and are usually not sporulated.

Blood

Fresh, heparinized, or citrated blood samples are best for examination. Delays reduce the chances of finding the parasites.

Place a drop of blood on a slide, overlay with a coverslip, and examine microscopically for living microfilariae or trypanosomes. Both groups of parasites are motile and can be

seen swimming among the formed blood elements. Motility is significantly decreased if the blood sample is refrigerated. If an organism is seen, the smears should be prepared and stained, preferably with Giemsa solution.

Urine

Gross Examination

Observe and record the degree of turbidity and the color of the specimen.

Microscopic Examination

1. Take a drop of urine with a Pasteur pipette, preferably from the bottom of the container, and transfer it to a glass slide.
2. Examine microscopically.
If *Trichomonas vaginalis* is suspected, the specimen must be fresh (<1 hour old), as the trophozoites quickly lose their characteristic morphology and motility.

Sedimentation by Centrifugation

1. Divide the entire urine specimen into 15 ml conical glass centrifuge tubes.
2. Sediment at 1000 rpm for 5 minutes.
3. Discard the supernatant.
4. Re-suspend the pellets with a Pasteur pipette and examine microscopically.

Sputum

Gross Examination

Observe and record the appearance of the specimen.

Microscopic Examination

1. Transfer a small amount of sputum with a wooden applicator stick to a clean glass slide.
2. Add a drop of normal saline solution.
3. Examine microscopically.

Sedimentation by Centrifugation

1. Mix sputum with equal parts of 3% NaOH.
2. Let stand 5 minutes.
3. Sediment at 1000 rpm for 5 minutes and examine microscopically.

Tissues

Place a small piece of tissue between two clean glass slides using forceps, press to flatten, then examine under a microscope.

To examine skin scrapings:

1. Place the scrapings on a clean glass slide.
2. Add a drop of normal saline solution and overlay with a coverslip.
3. Let stand 30 minutes.
4. Press the coverslip gently to break up the skin pieces and then examine microscopically.

Whole tapeworms must be carefully examined for the presence of a scolex. It is located at the narrowest end of the strobila. If only proglottids are available for observation, they must first be preserved in 10% formaldehyde. Then, the central uterus is injected with India ink using a 25-gauge needle. It is then placed between two glass slides, compressed, and examined with the aid of a dissection microscope. With taenia segments, the lateral branches on one side of the main uterine stem are counted (see *Taenia saginata* and *T. solium*). Arthropods are best identified preserved. The specimen should be placed in 70% ethanol and transferred to a Petri dish for examination when no longer motile.

Aspirated Fluids**Sedimentation by Centrifugation**

1. Centrifuge clear fluid aspirates at 1000 rpm for 5 minutes in a conical centrifuge tube.

2. Decant supernatant.
3. Examine the pellet microscopically.
4. Stain by the Wheatly-Gomori trichrome procedure.

Miscellaneous Tests**Tape test for *Enterobius vermicularis* (Pinworm)**

Clear tape preparations of various types, available commercially, are routinely used in the diagnosis of pinworm infection. The tape is placed with the sticky side down on the perineum, and eggs or adult worms adhere to it. The tape is then examined microscopically. Adult pinworms are also occasionally found on the surface of formed stool samples. Occasionally, eggs of *Taenia* spp. are seen on sticky tape tests.

Preserved Specimens

Whenever a delay of 24 hours or longer is anticipated, the specimen should be preserved. The preservative to be employed depends on the type of test selected.

Stool: Direct Smear

Merthiolate-iodine-formaldehyde Method (MIF). a solution of merthiolate, iodine, and formaldehyde (MIF) preserves and stains trophozoites and cysts. The organisms develop an orange color, but this stain does not last. Therefore, a permanent stain should also be done on the same stool sample. MIF is not acceptable for other staining procedures, such as the Wheatly-Gomori trichrome stain.

1. Emulsify 1 g of stool sample in 10 ml of MIF solution.
2. Place a drop of stool-MIF-emulsion on a clean glass slide and examine microscopically.

Stools preserved in MIF can be concentrated by sedimentation using the formaldehyde-ethyl acetate method.

Wheatly-Gomori Trichrome Stain for PVA-Preserved Stool

Stool specimens preserved in polyvinyl alcohol (PVA) can be stained by the Wheatly-Gomori trichrome method, which is the same as that for unpreserved stools, except that Schaudinn's fixative is not necessary and the staining time differs.

Solution	Time
Ethanol-iodine 70%	10–20 minutes
Ethanol 70%	3–5 minutes
Ethanol 70%	3–5 minutes
Trichrome stain	8–10 minutes
Ethanol 90% (acidified)	1–10 seconds

Dip the coverslip in the destaining solution once or twice. Rinse in 95% alcohol to stop the process. Thin smears destain quickly; thicker smears require three to five dips.

Solution	Time
Ethanol 95%	Rinse
Ethanol 95%	5 minutes
Xylol	10 minutes

Mount the stained coverslip and examine microscopically.

Blood

Microscopic Examination

A thick smear consists of several drops of blood on a slide, dried in air, and hemolyzed by immersion in a hypotonic solution. This process concentrates the parasites. A thin smear is prepared by making a film of blood analogous to that used for a differential count of the white cells. Giemsa staining is recommended for both preparations.

1. Immerse the slide in 100% ethanol or methanol for 2–3 minutes.
2. Make a solution consisting of 1 drop of concentrated Giemsa stain per 1 ml of distilled water (pH 7.4) and fill a Copeland jar with 50 ml of the mixture.
3. Stain for 10–30 minutes.
4. Wash in distilled H₂O.
5. Air dry the slide.
6. Examine microscopically under oil immersion. View 100 contiguous fields of a thin smear.

Concentration by Sedimentation: Knott Test

The Knott technique concentrates and preserves filarial microfilariae, which can be stained by Giemsa solution and identified morphologically.

1. Mix 1 ml of heparinized blood with 9 ml of 2% formaldehyde.
2. Centrifuge at 2,000 rpm for 10 minutes.
3. Decant the supernatant.
4. Examine the sediment microscopically.

If microfilariae are present, they are stained as follows:

1. Spread the sediment on a clean glass slide.
2. Dry overnight.
3. Stain with Giemsa solution (1 ml of concentrated Giemsa stain in 50 ml of distilled H₂O at pH 7.4).
4. Destain 10–15 minutes in H₂O.
5. Air dry.
6. Examine microscopically.

Solutions

Schaudinn's Fixative

- HgCl₂, saturated aqueous solution: 666 ml (add 80 g HgCl₂ to 1 liter de-ionized H₂O; stir 3–4 hours and then filter)

- Ethyl alcohol 95%: 333 ml
- Ethanol-iodine solution 70%. Add enough crystalline iodine to 70% ethanol to turn the solution deep amber-brown; filter before using.

Wheatly-Gomori Trichrome Stain

- Chromotrope 2R 0.6 g
- Light green SF 0.3 g
- Phosphotungstic acid 0.7 g

Mix with 1 ml of glacial acetic acid and stir gently for 20 minutes. Add 100 ml of distilled H₂O, then store in dark brown bottle.

Buffered Formaldehyde

- Formaldehyde solution 37–40% 100ml
- Sodium phosphate (monobasic, 4.0 g anhydrous)
- Sodium phosphate (dibasic, 6.5 g anhydrous)
- H₂O 900 ml

Adjust the pH of the solution to 7.0

Zinc Sulfate (ZnSO₄)

- ZnSO₄ 333 g
- H₂O (50–55°C) 1000 ml

Adjust the specific gravity to 1.18 by adding either more H₂O or more zinc sulfate crystals.

Sugar Solution (Sheather's Method)

- Sucrose 500 g
- H₂O 320 ml
- Phenol 6.5 g

Merthiolate-Iodine-Formaldehyde Solution

- Tincture of Merthiolate No. 99
1:1000 100 ml
- Formaldehyde solution 37–40%
25 ml
- Glycerol
5 ml
- H₂O
250 ml

Store solution in a dark bottle.

Lugol's Iodine Solution

- Iodine 5 g
- Potassium iodide 10 g
- H₂O 100 ml

Polyvinyl alcohol (PVA)

Polyvinyl alcohol is available commercially.

- Schaudinn's fixative 935 ml
- Glycerol 15 ml
- Glacial acetic acid 50 ml
- Polyvinyl alcohol (powder) 50 g
- H₂O 1000 ml



Veena Tandon, Ph.D. (born 1949)

Tandon is an Indian parasitologist whose contribution to science was acknowledged by the government of India awarding her the Padma Shri, their fourth highest civilian honor. Dr. Tandon is credited as the chief instigator of the North-East India Helminth Parasite Information Database (a critical database of parasite biodiversity for this region) and is the recipient of the Lifetime Achievement Award of the Indian Society of Parasitology for her work on helminths. Dr. Tandon has delivered many keynote addresses and has authored many texts and articles.

Appendix C: Diagnostic Color Atlas of Protozoa and Helminths

This atlas is intended as a pictorial reference for the diagnostic laboratory. It is important to be reminded that the laboratory obtains the most relevant information regarding a given parasitic infection. The physician must act according to the findings of the laboratory. Pattern recognition is the key to becoming a competent parasitology diagnostic technician. Space only permits a single example to be shown of each relevant stage of the major parasites infecting the human host. Our atlas can only serve as a guide for a much broader range of variation in both size and shape for any given diagnostic stage. When an object is encountered under the microscope, the parasitic stage usually looks as it is depicted here. Occasionally, even the most experienced laboratory technician may be unsure of, or express some doubt about the identity of some objects. A few commonly encountered artifacts are shown for comparison. Suggested readings to more comprehensive atlases are listed,

in case more visual examples are desired for comparison with the object in question. Internet resources for each parasite stage have become an invaluable aid for helping with identification. There is no shortcut to becoming familiar with each parasite. Only by co-observing patient samples with an accomplished technician can the skills necessary for advancement to the front lines of the diagnostic laboratory be developed.

It is very helpful to have a camera (preferably a digital image capturing device) attached to the diagnostic microscope. Images can then be stored on a computer and recalled on-demand. This allows for the accumulation of a permanent record of interesting objects encountered under the microscope. Such images are extremely helpful during training sessions for novice parasitology technicians. Digital images can be sent via email, permitting instant consultation with any expert group, such as the Centers for Disease Control (CDC) and Prevention, in Atlanta, Georgia.



Anton van Leeuwenhoek, microscopist *extraordinaire*. Discoverer of the trophozoite of *Giardia lamblia*.

The majority of the protozoa depicted here have been stained with either iron-hematoxylin (blue-gray stain) or Wheatly and Gomori's trichrome stain (green and red stain). Helminth eggs are as they appear in unstained, concentrated stool samples. Their yellow-brown tints attest to the fact that they have

encountered bile pigments. Microfilariae have all been stained with Giemsa (blue and red stain), as have the malaria parasites. The tissue section of the Nurse cell-parasite complex of *Trichinella spiralis* is stained with hematoxylin and eosin.

Protozoa



Figure C.1. *Giardia lamblia* trophozoite
15 μ m

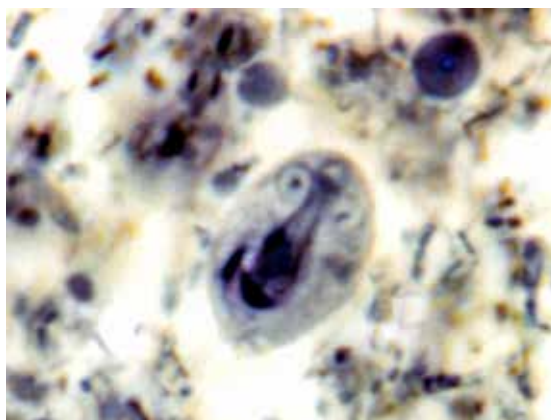


Figure C.2. *Giardia lamblia* cyst
15 μ m



Figure C.3. *Trypanosoma brucei rhodesiense*
25 μ m x 3 μ m

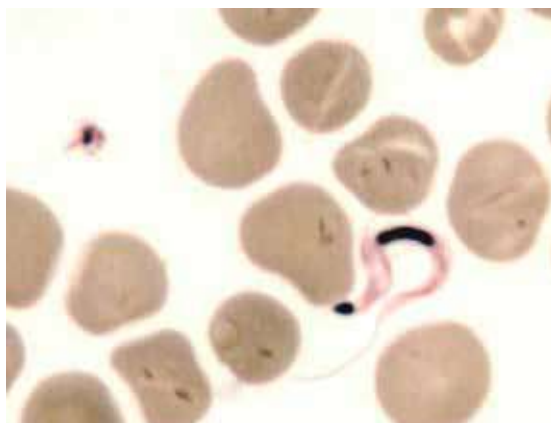


Figure C.4. *Trypanosoma cruzi*
20 μ m x 3 μ m

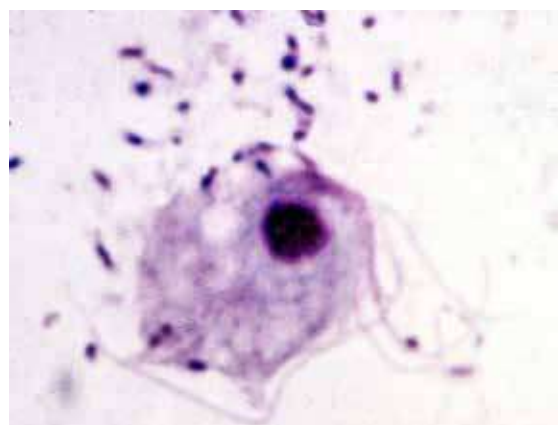


Figure C.5. *Trichomonas vaginalis*
20 μ m x 10 μ m



Figure C.6. *Plasmodium falciparum* ring stage



Figure C.7. *Plasmodium falciparum* macrogametocyte

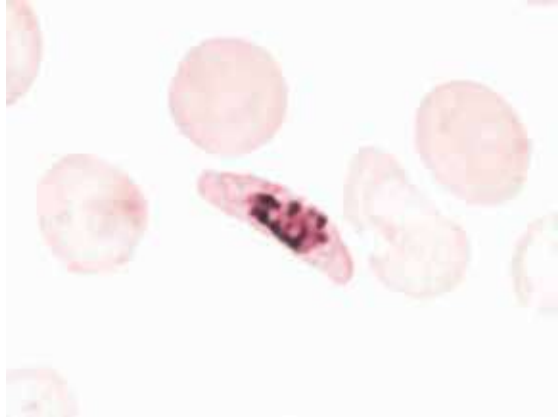


Figure C.8. *Plasmodium falciparum* microgametocyte

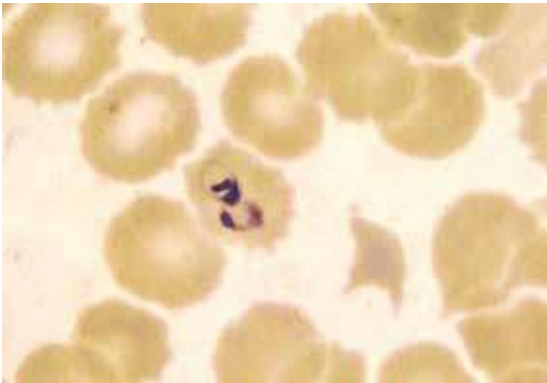


Figure C.9. *Plasmodium vivax* ring stage

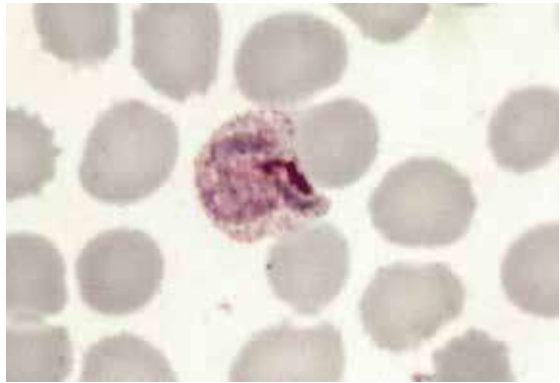


Figure C.10. *Plasmodium vivax* trophozoite

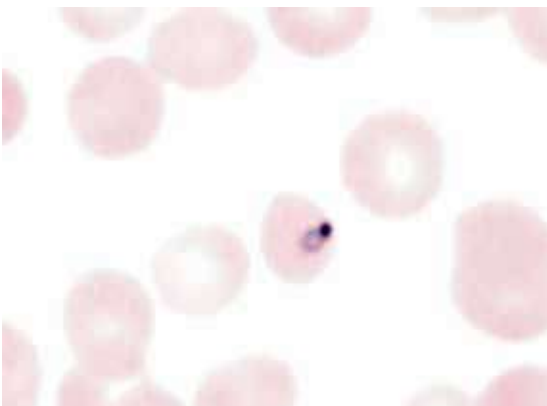


Figure C.11. *Plasmodium malariae* ring stage

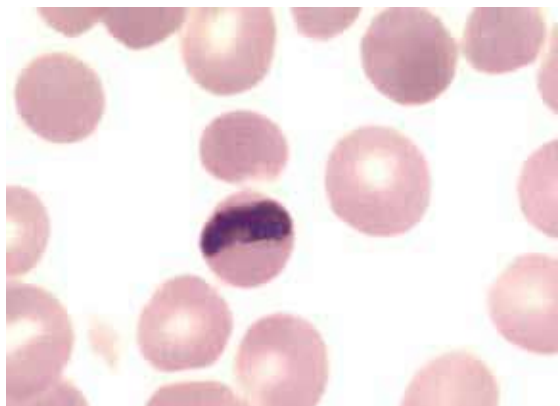


Figure C.12. *Plasmodium malariae* trophozoite



Figure C.13. *Cryptosporidium parvum* oocyst (acid-fast stain) 5 μ m

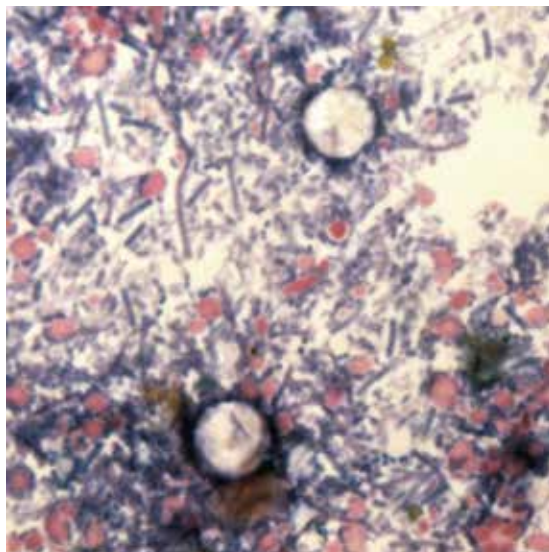


Figure C.14. *Cyclospora cayetanensis* 10 μ m

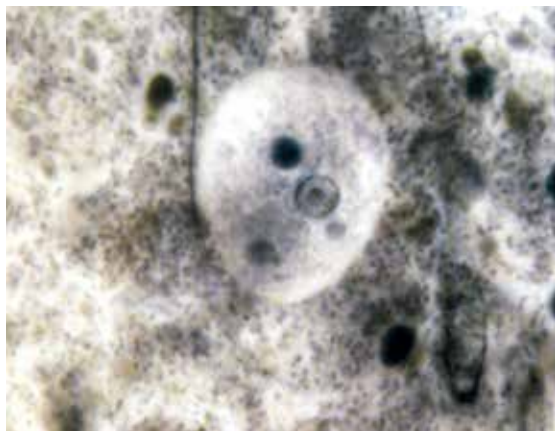


Figure C.15. *Entamoeba histolytica* trophozoite (note red cell in cytoplasm)

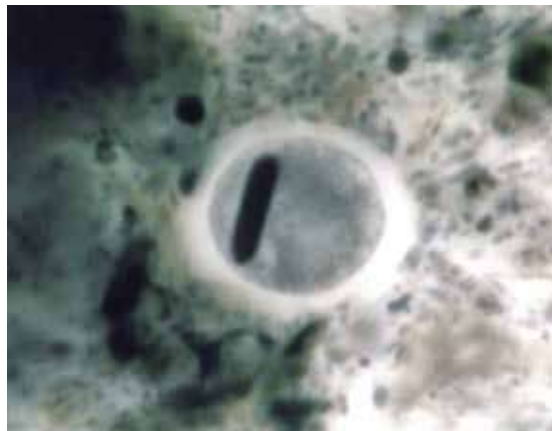


Figure C.16a. *Entamoeba histolytica* cyst
Note smooth-ended chromatoidal bar
15 μ m



Figure C.16b. *Entamoeba histolytica* cyst
(Through-focus #1): Note two nuclei
15 μ m

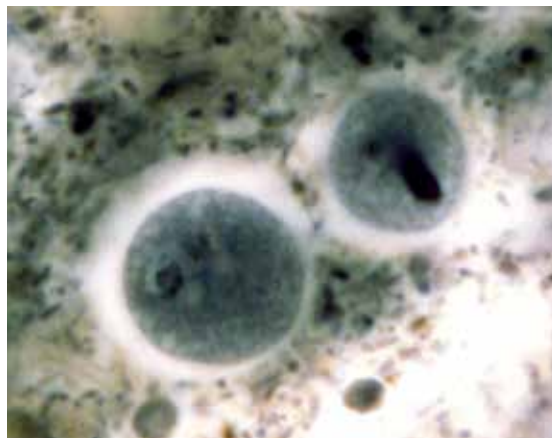


Figure C.16c. *Entamoeba histolytica* cyst
(Through-focus #2):
Note smooth-ended chromatoidal bar



Figure C.17. *Balantidium coli* trophozoite
150 μ m (unstained)

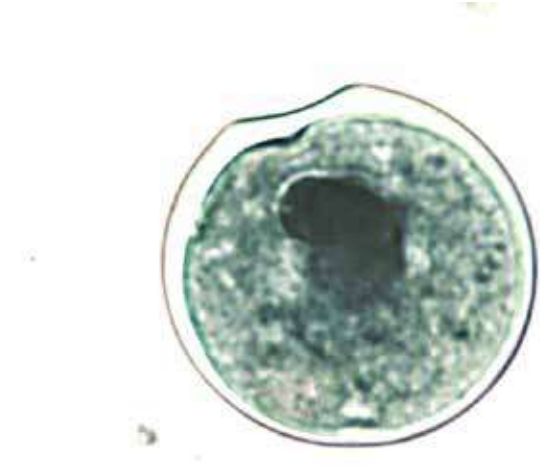


Figure C.18. *Balantidium coli* cyst
65 μ m

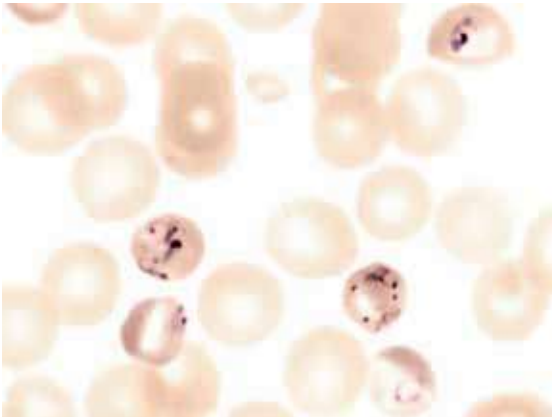


Figure C.19. *Babesia* spp. Bloodsmear

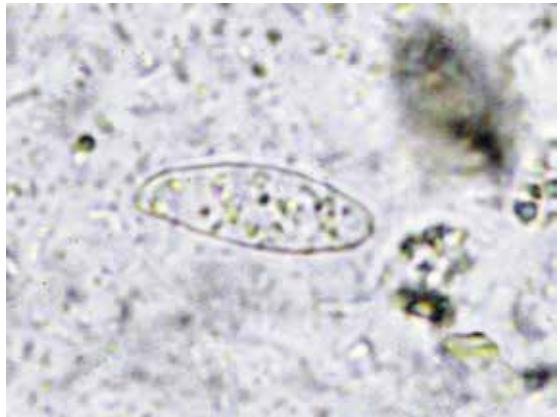


Figure C.20. *Cytoisospora belli* unsporulated
oocyst
25 μ m x 15 μ m



Figure C.21. *Cytoisospora belli* sporulated oocyst
25 μ m x 15 μ m

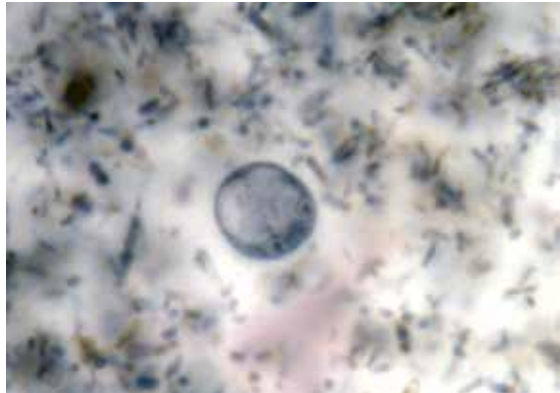


Figure C.22. *Blastocystis hominis*
6 μ m



Figure C.23. *Trichomonas hominis*
10 μm x 8 μm



Figure C.24. *Trichomonas tenax*
7 μm x 3 μm



Figure C.25. *Retortamonas hominis*
6 μm x 2 μm

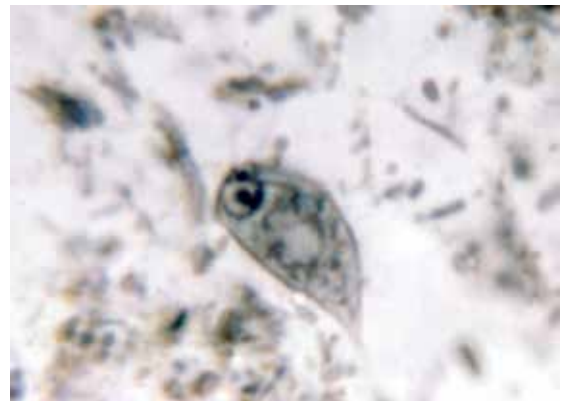


Figure C.26. *Chilomastix mesnili* trophozoite
15 μm x 12 μm

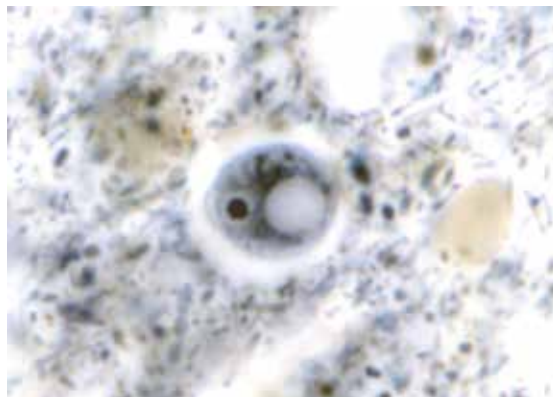


Figure C.27. *Chilomastix mesnili* cyst
8 μm x 5 μm

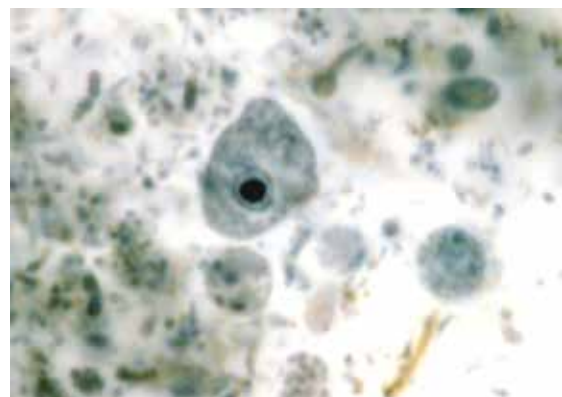


Figure C.28. *Endolimax nana* trophozoite
10 μm x 4 μm

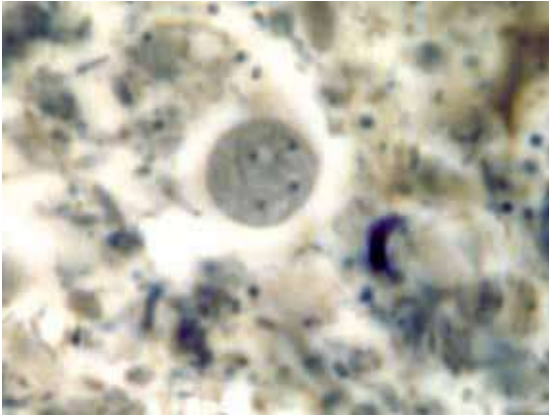


Figure C.29. *Endolimax nana* cyst
Note four nuclei
8 μ m x 6 μ m

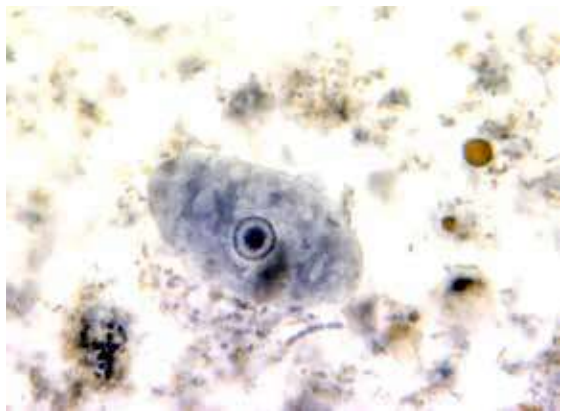


Figure C.30. *Iodamoeba bütschlii* trophozoite
18 μ m

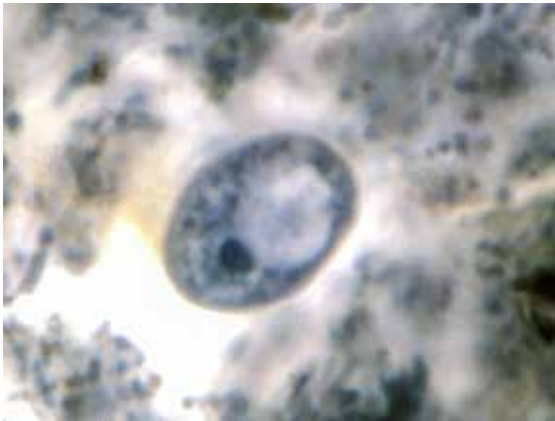


Figure C.31. *Iodamoeba bütschlii* cyst
12 μ m x 8 μ m



Figure C.32. *Entamoeba gingivalis* trophozoite
30 μ m

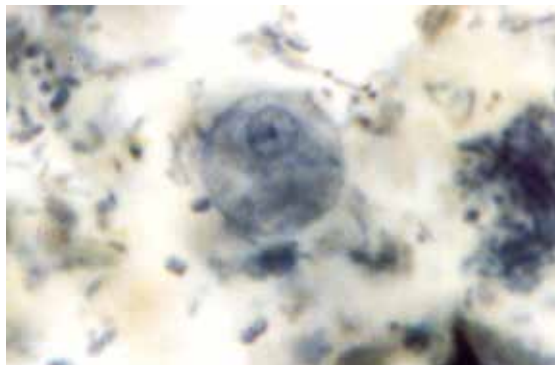


Figure C.33. *Entamoeba hartmanni* trophozoite
10 μ m



Figure C.34. *Entamoeba coli* trophozoite
35 μ m



Figure C.35a. *Entamoeba coli* cyst
(Through-focus #1): Two nuclei can be seen
30 μ m

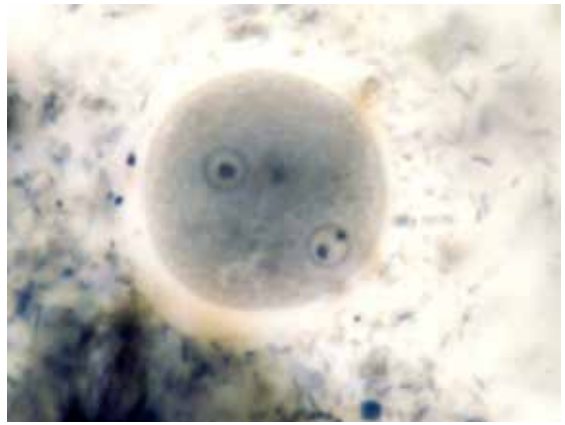


Figure C.35b. *Entamoeba coli* cyst
(Through-focus #2): Two nuclei can be seen
30 μ m



Figure C.35c. *Entamoeba coli* cyst
(Through-focus #3): Three nuclei can be seen
30 μ m

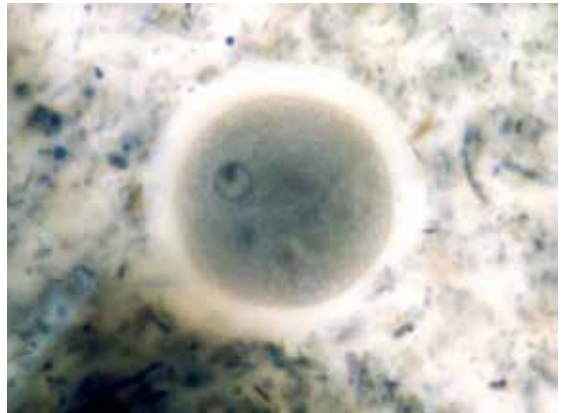


Figure C.35d. *Entamoeba coli* cyst
(Through-focus #4): One nucleus can be seen
A total of eight nuclei are present in the cyst
30 μ m

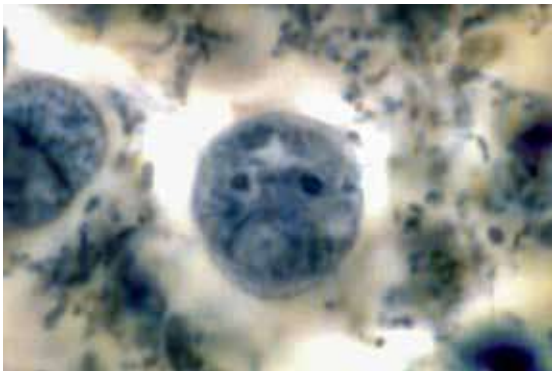


Figure C.36. *Dientamoeba fragilis* cyst
10 μ m

Nematodes



Figure C.37. *Enterobius vermicularis* ova
55 μm x 25 μm



Figure C.38. *Trichuris trichiura* ovum
50 μm x 20 μm



Figure C.39. *Ascaris lumbricoides* ovum
(fertilized)
60 μm x 35 μm



Figure C.40. *Ascaris lumbricoides* ovum
(fertilized, decorticated)
50 μm x 30 μm



Figure C.41. *Ascaris lumbricoides* ovum
(unfertilized)
Size variable; 70 μm x 30 μm



Figure C.42. Hookworm ovum
70 μm x 40 μm



Figure C.43. *Strongyloides stercoralis*
(rhabditiform larva)
500 μm x 15 μm



Figure C.44. *Strongyloides stercoralis*
(rhabditiform larva)
Note short buccal cavity (arrow)

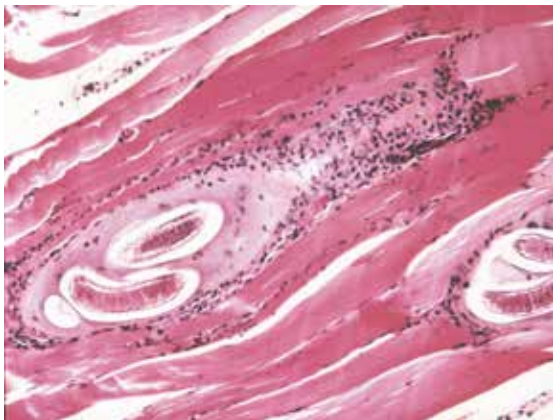


Figure C.45. *Trichinella spiralis*
Larvae in Nurse-cell in muscle



Figure C.46. *Wuchereria bancrofti* microfilaria
260 μm x 9 μm

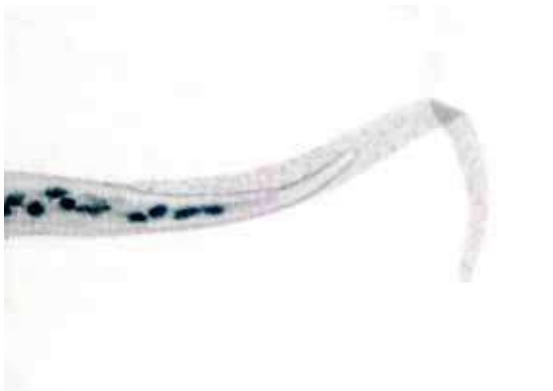


Figure C.47. *Wuchereria bancrofti* microfilaria
Note sheath and nuclei, which do not extend to end
of tail



Figure C.48. *Brugia malayi* microfilaria
200 μm x 6 μm



Figure C.49. *Brugia malayi* microfilaria
Note nucleus at tip of tail and sheath



Figure C.50. *Loa loa* microfilaria
35 mm x 40 μ m



Figure C.51. *Loa loa* microfilaria
Note sheath and nuclei, which extend to end of tail



Figure C.52. *Mansonella ozzardi* microfilaria
190 μ m x 4 μ m

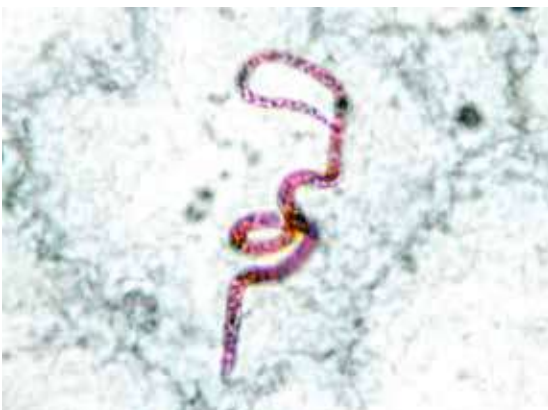


Figure C.53. *Mansonella perstans* microfilaria
200 μ m x 4 μ m



Figure C.54. *Capillaria philippinensis* ovum
40 μ m x 20 μ m



Figure C.55. *Capillaria hepatica* ovum
60 μm x 30 μm

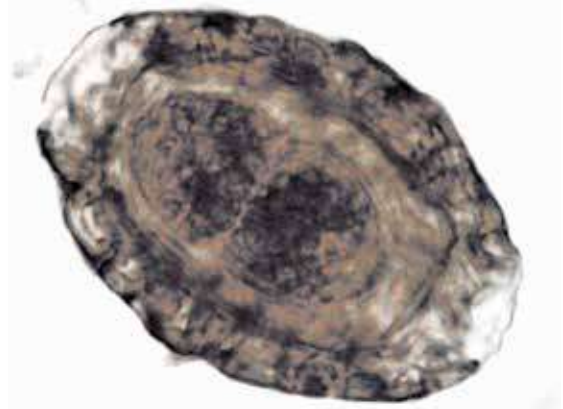


Figure C.56. *Dioctophyma renale* ovum
70 μm x 45 μm

Cestodes



Figure C.57. *Taenia* spp. ovum
40 μm x 30 μm



Figure C.58. *Diphylobothrium latum* ovum
65 μm x 45 μm



Figure C.59. *Dipylidium caninum* egg cluster
Each egg measures 35 μm



Figure C.60. *Hymenolepis nana* ovum
45 μm x 30 μm

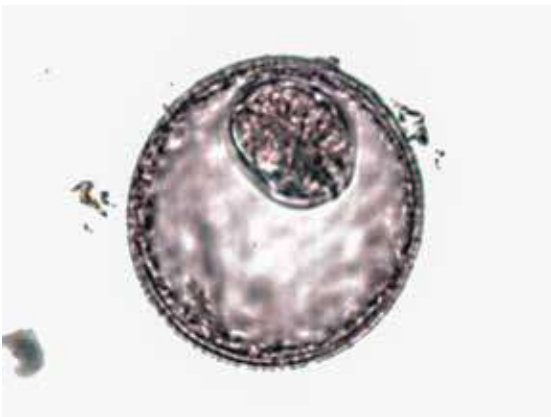


Figure C.61. *Hymenolepis diminuta* ovum
75 μm x 70 μm

Trematodes

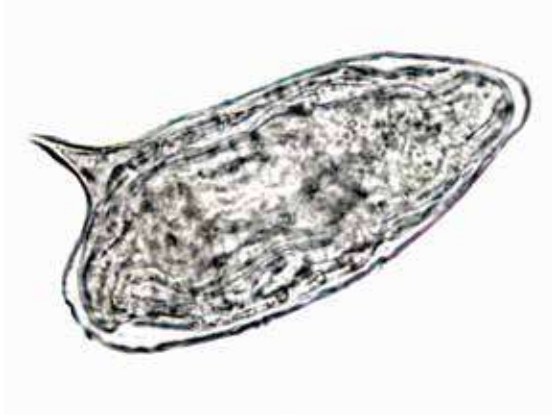


Figure C.62. *Schistosoma mansoni* ovum
160 μm x 60 μm



Figure C.63. *Schistosoma mansoni*
miracidium hatching from egg

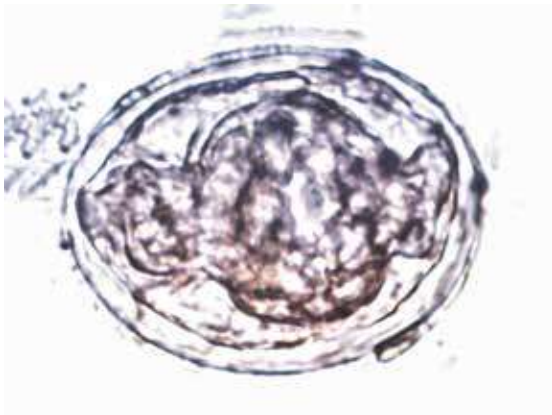


Figure C.64. *Schistosoma japonicum* ovum
85 μm x 60 μm



Figure C.65. *Schistosoma haematobium* ovum
170 μm x 60 μm



Figure C.66. *Schistosoma mekongi* ovum
60 μm x 45 μm



Figure C.67. *Fasciolopsis buski* ovum
135 μm x 80 μm



Figure C.68. *Fasciola hepatica* ovum
140 μm x 75 μm



Figure C.69. *Paragonimus westermani* ovum
110 μm x 60 μm



Figure C.70. *Clonorchis sinensis* ovum
30 μm x 16 μm

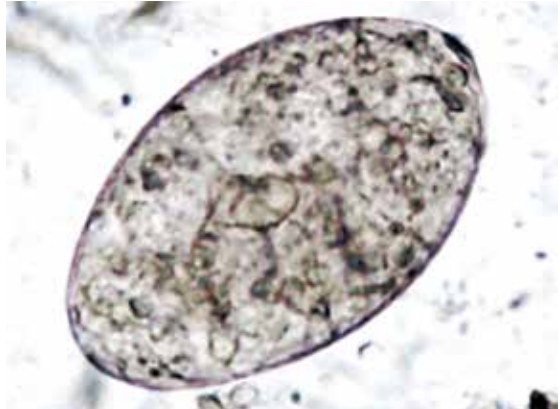


Figure C.71. *Echinostoma ilocanum* ovum
130 μm x 60 μm



Figure C.72. *Heterophyes heterophyes* ovum
20 μm x 15 μm

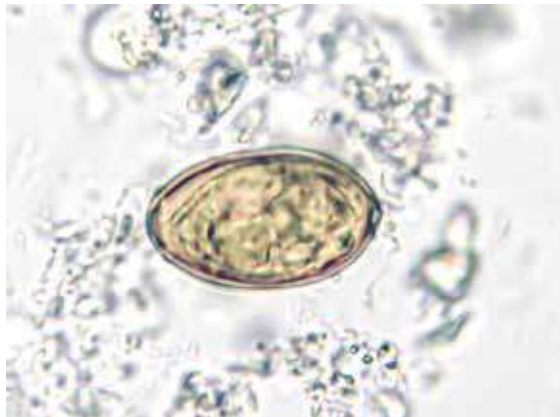


Figure C.73. *Metagonimus yokogawai* ovum
30 μm x 15 μm

Miscellaneous



Figure C.74. *Macrocanthorynchus hirudinaceus*
ovum
60 μm x 20 μm



Figure C.75. Charcot-Leyden crystal
10 μm x 2 μm



Figure C.76. Digested vegetable matter
(Artifact)

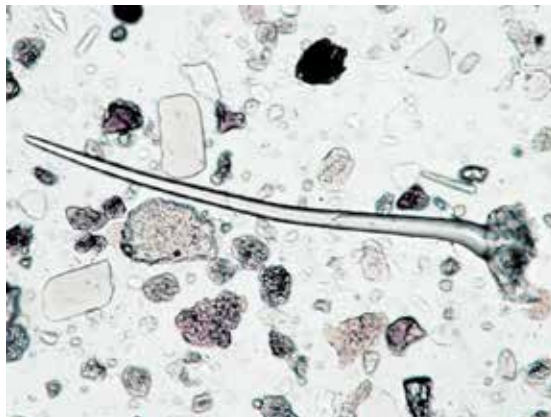


Figure C.77. Plant fiber
(Artifact)



Figure C.78. Pollen grain
(Artifact)

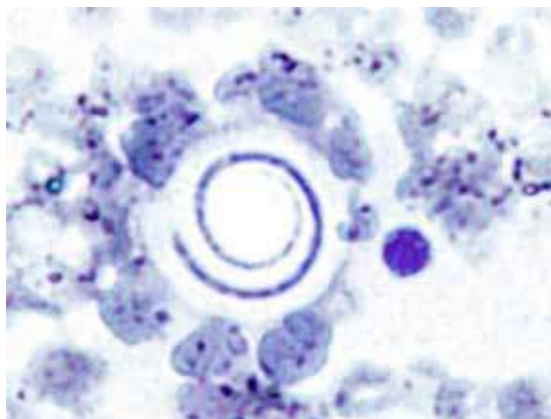
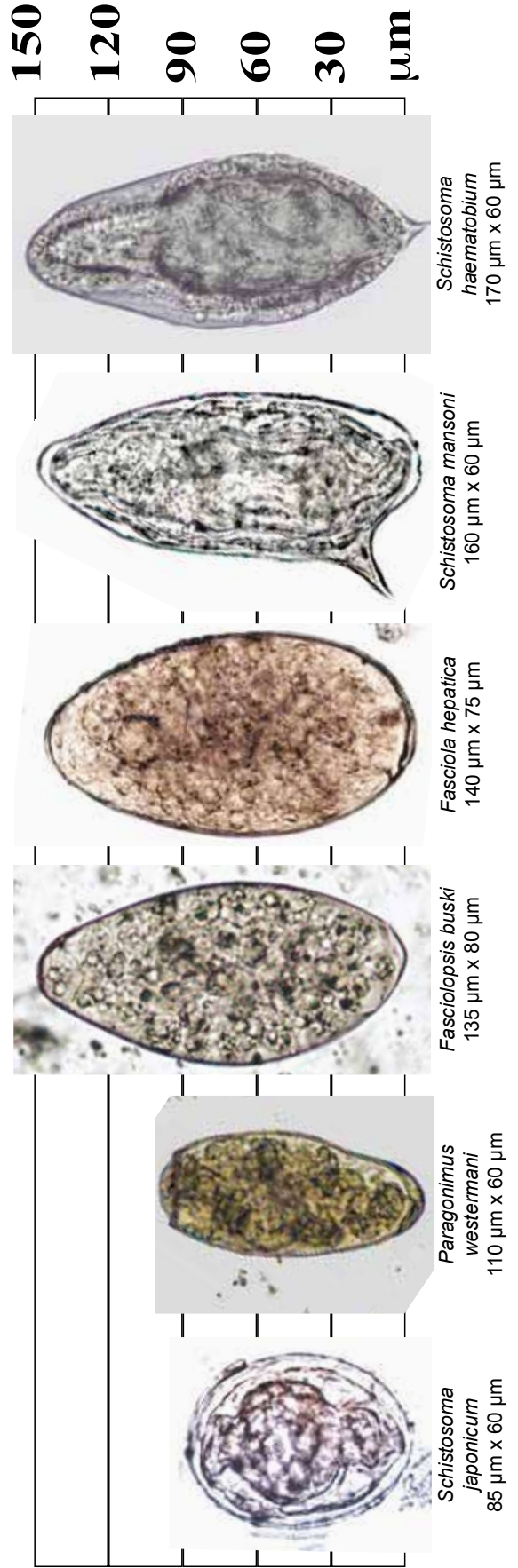
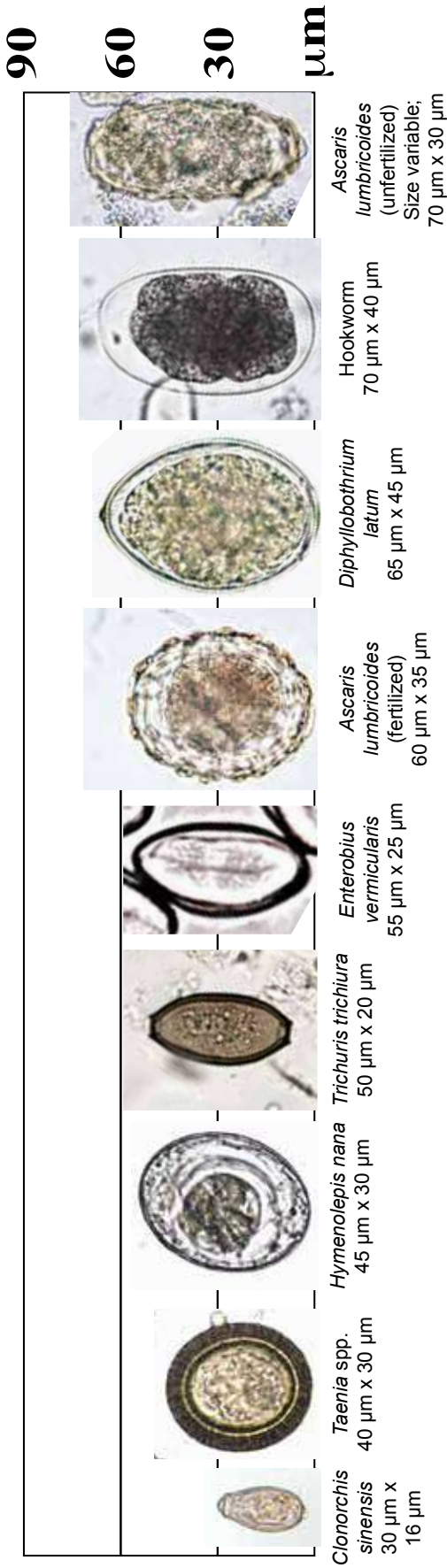


Figure C.79. *Helicosporium* spp.
(Fungus)



Rudolf Ludwig Carl Virchow, M.D. (1821–1902)

Virchow (\FIR-kow\, \fir-kō\) is the poster child for the saying: “No one is perfect”. To his long list of credits, he single-handedly established the field of anatomic pathology and was a passionate advocate of the use of microscopy for describing pathological conditions. His insatiable curiosity led him to investigate the life cycle of *Trichinella spiralis*. Virchow proved that the infection was transmitted from animal to animal by the ingestion of raw meat that harbored the infective larvae. He further determined that if the meat was heated to 137 °F for ten minutes, the infective larvae were killed, and the meat could then be eaten without medical consequences. He proved that humans became sick when they ingested infected raw or undercooked meat and helped to establish a meat inspection program aimed at eliminating the infection. He described numerous pathological conditions in humans, many of which still bear his name. On the negative side, Virchow was a staunch opponent of Darwin’s theory of evolution. He was also an opponent of the germ theory of disease, despite his work on *Trichinella spiralis*. He died holding onto these beliefs.





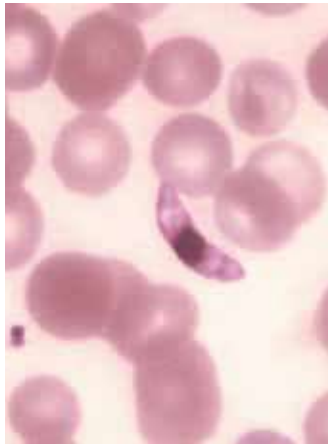
Wuchereria bancrofti microfilaria
260 µm x 9 µm



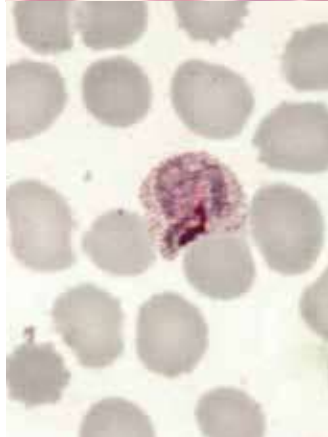
Wuchereria bancrofti microfilaria. Note sheath and nuclei, which do not extend to end of tail



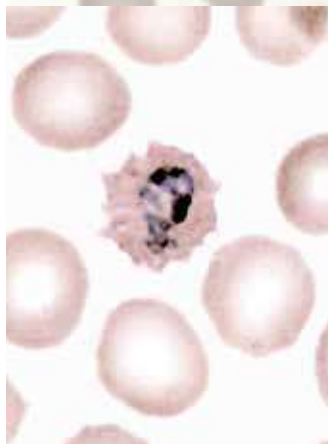
Loa loa microfilaria. Note sheath and nuclei, which extend to end of tail



Gametocyte of *Plasmodium falciparum*.



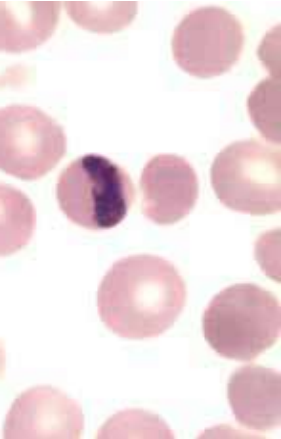
Trophozoite of *P. vivax*. Note Schüffner's dots in the parasite, and surrounding red cells that are smaller than the infected one.



Trophozoite of *P. ovale*. Note "crenated" appearance of infected red cell. Courtesy M. Guelpe.



Signet ring stage of *Plasmodium* spp.



Plasmodium malariae trophozoite.



Marietta Voge Ph.D. (1918-1984)

Marietta Voge was a leader in the field of parasitology education. Marietta was the co-author of one of the leading textbooks in the field and served as the president of the American Society of Parasitologists in 1976. Dr. Voge not only inspired many future parasitologists but she also devoted her time to working in many resource-limited parts of the world, refugee camps and mission clinics. She is well remembered for her dedication to teaching, and her life was an embodiment of her words from her presidential address to the American Society of Parasitologists where she wrote: “It is the teacher that sparks and stimulates the minds of students to produce new ideas. It is the teacher who gives the impetus to students to become the most important propellant to our society and civilization - the innovator – because, without innovation and originality, society becomes stagnant and decadent. A superior teacher is the interpreter and keeper of our past, the critic of our present, the dreamer of things to come.”



Otto Edward Henry Wucherer, M.D. (1820–1873)

Wucherer is best remembered for his clinical description of severe anemia caused by hookworm infection, and his description of the microfilariae of the nematode parasite, *Wuchereria bancrofti*, that he obtained from the urine of his infected patients. He shares the name of the filarial parasite with Joseph Bancroft.

Pronunciation assistance

A pronunciation guide is provided for readers requiring clarification of the pronunciation of individual terms or words. Below are instructions on use and interpretation to best facilitate accurate pronunciation.

Two forms of pronunciation assistance are provided, with the American IPA (International Phonetic Alphabet) typically provided at the chapter heading and the pronunciation guide given within the text, e.g., for *Clonorchis* klō- 'nôr-kəs (American IPA) and KLO-nor-kis (pronunciation guide).

If the pronunciation is provided within the text, then the pronunciation guide information comes first and the American IPA second. Separate terms are provided between two backslashes, e.g., for *Clonorchis* \KLO-nor-kis\ (pronunciation guide), and \klō- 'nôr-kəs\ (American IPA).

Each word or term is broken up into manageable components or syllables and separated by a hyphen. The hyphens are placed according to phonetic principles of the spoken word where speech has been slowed down to facilitate a model or example of pronunciation.

Pronunciation guide

In the pronunciation guide, each syllable for the selected word is written in lower case (abc) or upper case (ABC). The capitalized syllables represent the stressed part of a word or the part of a word pronounced the loudest, in comparison to the other syllables pronounced more quietly and therefore not stressed or capitalized.

Some English words have tricky spelling and pronunciation conventions and therefore are written phonetically (how they sound) in the pronunciation guide and not necessarily as they are spelled. For example, the word “physics” would appear as \fizz-iks\. In this case, there is no stress on either of the syllables and therefore no capital letters were used, only lower case. However, the word “atom” would appear as \at-OM\. In this case, the word also has two syllables, but there is stress on the second syllable, and therefore it is capitalized.

American IPA (International Phonetic Alphabet)

The second pronunciation tool is the American International Phonetic Alphabet. This alphabet is based on the International Phonetic Alphabet (IPA) and is specifically adapted to suit the needs of speakers of the general American variety. The IPA is a standardized phonetic notation of the alphabet designed to assist readers with the spoken version or pronunciation of a word. Below is a table that provides all the symbols in IPA, as well as American IPA, the sound name, and examples of common American English words to exemplify that sound.

IPA symbol	American IPA symbol	Sound name	Examples
eɪ	ā	long a	<u>l</u> ake, pa <u>y</u> , ei <u>g</u> ht, pai <u>n</u>
æ	a	short a	<u>f</u> at, pa <u>t</u> , da <u>d</u> , ca <u>t</u>
i	ē	long e	<u>b</u> ee, ke <u>e</u> p, ea <u>t</u> , hea <u>t</u>
ɛ	e	short e	be <u>d</u> , pe <u>t</u> , pe <u>pp</u> er, de <u>s</u> k, fe <u>t</u> ch
aɪ	ī	long i	hi <u>k</u> e, pi <u>e</u> , by, hi <u>g</u> h, tr <u>y</u>
ɪ	i	short i	fi <u>t</u> , pi <u>t</u> , i <u>t</u> , di <u>g</u> , dri <u>n</u> k, sy <u>s</u> tem
oʊ	ō	long o	ho <u>m</u> e, to <u>e</u> , do <u>u</u> gh, o <u>h</u> , bo <u>a</u> t
ɑ	‘ā	short o	mo <u>p</u> , po <u>t</u> , ho <u>t</u> , bo <u>d</u> y
ʌ	‘ə	short u	bu <u>t</u> , bu <u>d</u> , cu <u>p</u> , flo <u>o</u> d, so <u>n</u>
ʊ	ù	other u	pu <u>ll</u> , wo <u>l</u> f, wo <u>o</u> d, go <u>o</u> d, pu <u>t</u> , co <u>u</u> ld
u	ü	oo sound	spoo <u>n</u> , tw <u>o</u> , sho <u>o</u> t, i <u>n</u> flu <u>e</u> nce, mo <u>o</u> ve, do
ɔ	ò	aw sound	pa <u>w</u> , wa <u>l</u> k, ta <u>l</u> k, sa <u>w</u> , Pa <u>u</u> l
ɔɪ	òi	oi sound	jo <u>i</u> n, co <u>i</u> n, to <u>y</u>
aʊ	aù	ow sound	ho <u>w</u> , vo <u>w</u> , bo <u>w</u> , ho <u>u</u> se
a:	ā	ar sound	ca <u>r</u> , da <u>r</u> k, pa <u>r</u> k, fa <u>r</u> ther
ɔr	ùr	or sound	sto <u>r</u> e, po <u>o</u> r, to <u>u</u> r
ɛr	‘e	air sound	pa <u>i</u> r, ca <u>r</u> e, the <u>r</u> e
ɜ	ər	er sound	wo <u>r</u> k, we <u>r</u> e, bi <u>r</u> d, di <u>r</u> t, nu <u>r</u> se, sti <u>r</u> , co <u>u</u> rage
ə	ə	*schwa	a <u>b</u> out, a <u>g</u> o, fa <u>m</u> ous, o <u>c</u> ea <u>n</u>
b	b	b sound	<u>b</u> aby
tʃ	ch	ch sound	<u>ch</u> ease
d	d	d sound	<u>d</u> ig
f	f	f sound	<u>f</u> ace
g	g	g sound	<u>g</u> ift
h	h	h sound	<u>h</u> at
dʒ	j	j sound	<u>j</u> ump
k	k	k sound	<u>c</u> at
l	l	l sound	<u>l</u> ike
m	m	m sound	<u>m</u> e
n	n	n sound	<u>n</u> ot
ŋ	ng	ng sound	<u>sing</u>
p	p	p sound	<u>p</u> ig
r	r	r sound	<u>r</u> ed
s	s	s sound	<u>s</u> ome
ʃ	sh	sh sound	<u>she</u>
t	t	t sound	<u>t</u> ime
θ	th	unvoiced th	<u>th</u> ink
ð	th	voiced th	<u>th</u> em
v	v	v sound	<u>v</u> ery
w	w	w sound	<u>w</u> ater
y	y	y sound	<u>y</u> es
z	z	z sound	<u>z</u> ip
ʒ	zh	zh sound	<u>u</u> sual

*The symbol [ə] is called a “schwa”. It is only used to represent unstressed syllables.

Name	Pronunciation Guide	American IPA
<i>Acanthamoeba</i>	ah-KAN-thah-MEE-bah	ə-ˌkanth-ə-ˈmē-bə
<i>Adenocephalus</i>	add-no-SEF-a-lus	ə-ˈde-nə-ˈsefələs
<i>Ancylostoma</i>	an-see-LOS-tow-mah	an-sə-ˈlās-tə-mə
<i>Angiostrongylus</i>	AN-jee-o-STRON-jah-lus	ˈan-jē-ə-ˈsträn-jə-ləs
<i>Anisakis</i>	an-ee-SAY-kiss	a-nə-ˈsā-kəs
Antoni	an-TOON-nee	an-ˈtō-nē
<i>Ascaris</i>	ASS-ka-ris	ˈas-kə-rəs
<i>Babesia</i>	bah-BEE-zh-ah	bə-ˈbē-zh-ə
<i>Balamuthia</i>	BAL-a-moo-thee-ah	
<i>Balantidium</i>	BAL-an-TID-ee-um	bal-ən-ˈtid-ē-əm
<i>bancrofti</i>	ban-KROFT-ee	ban-ˌkrôft-ī
<i>Baylisascaris</i>	BAY-lis-as-KA-RIS	
Bignami	BEN-yah-meh	
<i>Blastocystis</i>	BLAS-tow-sis-TIS	
<i>brucei</i>	BREW-see	brūˈsē-ī
<i>cantonensis</i>	KAN-ton-en-SIS	
<i>ceylanicum</i>	SCI-la-nee-cum	
<i>Chagas</i>	SHAh-gas	chägəs
<i>Chrysops</i>	KRIS-ops	kris-ˌäps
<i>Clonorchis</i>	KLO-nor-kis	klō-ˈnôr-kəs
<i>coli</i>	KOHL-eye	kō-ˌlī
<i>costaricensis</i>	KOS-tar-EE-sen-sis	
<i>Cryptosporidium</i>	krip-tow-SPOR-i-dee-um	krip-tō-spôr-ˈi-dē-əm
<i>Cyclospora</i>	SCI-KLO-spor-ah	sī-klō-ˈspôr-ə
<i>dendriticum</i>	DEN-drit-IK-um	den-ˈdri-tik-əm
<i>Dientamoeba</i>	DYE-ant-ah-mee-bah	dī-ˌent-ə-ˈmē-bə
<i>Diphyllobothrium</i>	DYE-fil-ow-both-REE-um	dīˌfil-ə-bäth-rē-əm
<i>Dipylidium</i>	DYE-pie-LID-ee-um	dī-ˌpī-ˈlid-ē-əm
<i>dirofilaria</i>	DYE-row-fi-LAR-ee-a	dī-rō-fə-ˈlar-ē-ə
<i>Dracunculus</i>	dra-KUNG-kyoo-lus	drə-ˈkəŋ-kyə-ləs
<i>Echinococcus</i>	eh-KYE-no-KOK-us	i-ˌkī-nə-ˈkă-kəs
<i>Echinostoma</i>	eh-KYE-no-STO-mah	i-ˌkī-nə-ˈstō-mə
<i>Endolimax</i>	en-DOW-lie-MAKS	ˈen-də-ˈlī-ˌmaks
<i>Entamoeba</i>	ent-a-MEE-ba	en-tə-ˈmē-bə

Name	Pronunciation Guide	American IPA
<i>Enterobius</i>	EN-ter-owe-BEE-us	ent-ə- 'rō-bē-əs
<i>Enteromonas</i>	EN-ter-owe-MOW-nas	ent-ə- 'rō- 'mō- ,nas
<i>falciparum</i>	fal-SIP-pah-rum	fal- 'si-pə-rəm
<i>Fasciola</i>	fah-SEE-ow-lah	fə- 'sē-ə-lə
<i>Fasciolopsis</i>	FAH-see-ow-lop-sis	'fā-shō 'lāp-səs
<i>gambiense</i>	gam-BEE-in-ss	gam-bē-en'sē
<i>Giardia</i>	jee-ARE-dee-ah	jē-är'dē-ə
<i>Gnathostoma</i>	nah-THOS-tah-mah	nə- 'thās-tə-mə
<i>gondii</i>	gon-DEE	gon'dē-ī
<i>haematobium</i>	HEE-mah-tow-BEE-um	hē-mə- 'tō-bē-əm
<i>Hemiptera</i>	heh-MIP-tah-rah	hi- 'mip-tə-rə
<i>Heterophyes</i>	HET-er-OW-fye-eez	'he-tə- ,rō'fī-ēz
<i>histolytica</i>	HIS-tow-lit-i-ka	his'təlit'ikə
<i>Hymenolepis</i>	HI-men-OL-oh-pis	hī-mə- 'nāl-ə-pəs
<i>kellicotti</i>	KEL-lee-KOT-tee	
<i>lamblia</i>	lam-BLEE-ah	lām'blē-ə
<i>lanceolatum</i>	LAN-see-o-LATE-um	'lan-sē-ə- ,lāt-əm
<i>Laveran</i>	LAH-ver-RAH	la-və- 'rā ⁿ
Leeuwenhoek	LAY-vun-HOOK	lā-vən- ,hūk
<i>Leishmania</i>	LEESH-ma-NEE-ah	lēsh- 'ma-nē-ə
<i>Loa</i>	low-ah	lo'ə
<i>lumbricoides</i>	lum-BRI-koy-dz	'ləm-bri-kōi-dz
<i>malariae</i>	ma-ler-EE-ay	mə'lerē ,ē
<i>malayi</i>	MAH-la-i	
<i>Mansonella</i>	MAN-so-nel-ah	man-sə- 'nel-ə
<i>mansoni</i>	MAN-sow-nigh	man-sə- ,nī
<i>medinensis</i>	MEH-dee-NEN-sis	
<i>Mesocostoides</i>	MEH-zo-SES-toy-DEEZ	me-zə-ses- 'tōi-dēz
<i>moshkovskii</i>	mosh-KOV-ski-ee	
<i>Naegleria</i>	NAY-glir-EE-ah	nā- 'glir-ē-ə
<i>Nanophyetus</i>	NAH-no-FIE-ee-tus	nānə ,fī'ētəs
<i>Necator</i>	ne-KAY-tor	nə- 'kāt-ər
<i>Oesophagostomum</i>	IS-of-ah-GOS-tow-mum	i- ,säf-ə- 'gās-tə-məm
<i>Onchocerca</i>	ONG-ko-ser-kah	äng-kə- 'sər-kə

Name	Pronunciation Guide	American IPA
<i>Opisthorchis</i>	AH-pis-thor-kis	äp-əs- 'thôr-kəs
<i>ovale</i>	OO-va-leh	ō- 'va-lē
<i>ozzardi</i>	OZ-zar-DEE	
<i>Paragonimus</i>	par-ah-GON-i-mus	par-ə- 'gän-ə-məs
<i>parvum</i>	PAAR-vum	pahr'vūm
<i>Plasmodium</i>	plaz-MODE-ee-um	plaz- 'mō-dē-əm
<i>procyonis</i>	pro-SCI-on-is	
<i>rhodesiense</i>	row-DEE-zan-ss	rō-dē-zē-en'sē
<i>Schistosoma</i>	SHIS-tih-sow-mah	shis-tə- 'sō-mə
<i>Simulium</i>	seh-MYOO-LEE-um	si- 'myü-lē-əm
<i>sinensis</i>	SCI-nen-sis	
<i>spinigerum</i>	SPI-ni-G'E-rum	
<i>stenocephala</i>	ste-NO-seh-FAAL-ah	ste-nō-sə 'fāl-ə
<i>streptocerca</i>	STREP-tow-ser-ka	strep-tə- 'sər-kə
<i>Strongyloides</i>	STRON-ji-LOI-deez	strän-jə- 'lòi- ,dēz
<i>suis</i>	SW-ee	sw ^{vee}
<i>suum</i>	SO-om	sù-ùm
<i>Taenia</i>	TEE-nee-ah	tē-nē-ə
<i>Ternidens</i>	ter-nah-DENZ	tər-nə- ,denz
<i>Terranova</i>	TEAR-ah-now-vah	ter-ə- 'nō-və
<i>Toxocara</i>	TOK-so-ka-rah	täk-sə- 'kar-ə
<i>Toxoplasma</i>	TOK-so-plaz-ma	täk-sə- 'plaz-mə
<i>Trichinella</i>	TRIK-in-el-ah	trik-ə- 'nel-ə
<i>trichiura</i>	trick-ee-UR-ah	
<i>Trichomonas</i>	trick-oh-MOAN-us	tri-kə- 'mō-nəs
<i>Trichuris</i>	TRICK-you-ris	trik- 'yür-əs
<i>Trypanosoma</i>	tri-PAN-oh-so-mah	tri- ,pan-ə- 'sō-mə
<i>Uncinaria</i>	un-sin-NAR-EE-ah	ən-sə- 'nar-ē-ə
<i>vaginalis</i>	vaj-gi-NAL-is	vaj-ə- 'nā-ləs
<i>Virchow</i>	FIR-kow	fir-kō
<i>vivax</i>	VYE-vax	vī- ,vaks
<i>volvulus</i>	VOL-view-lus	väl-vyə-ləs
<i>Welch</i>	WELL-ch	welch
<i>Wuchereria</i>	VOOK-ah-rer-EE-ah	vük-ə- 'rir-ē-ə

Bibliography

Pronunciations are based on those in everyday use as well as the standard references listed below. In a number of cases there are several acceptable pronunciations, in such instances, one of the many variations was chosen.

PMI. (n.d.) Farlex Partner Medical Dictionary. (2012). Retrieved October 11 2018 from <http://medical-dictionary.thefreedictionary.com/PMI>

Whonamedit.com. (2012). *Whonamedit - dictionary of medical eponyms*. [online] Available at: <http://www.whonamedit.com/doctor.cfm/2761.html>

Morris, W. (1969). *American heritage dictionary of the English language*. American heritage.

Virginia P. Studdert, Clive C. Gay and Douglas Blood Saunders *Comprehensive Veterinary Dictionary 3rd Edition*. Philadelphia: Saunders.

Miller-Keane, O. T. M., & O'Toole, M. T. (2003). Miller-Keane encyclopedia and dictionary of medicine, nursing, and allied health. 7th ed. Philadelphia: Saunders.

Dictionary, A. H. (2007). The American Heritage Medical Dictionary. Boston : Houghton Mifflin Co

Saunders, J. H. (2007). *Dorland's Medical Dictionary for Health Consumers*. Philadelphia: Saunders.

Mosby (2009) Mosby's Medical Dictionary: 8th edition. Elsevier Health Sciences
Stedman's Medical Dictionary for the Health Professions and Nursing Seventh edition
2012 Wolters Kluwer Health | Lippincott Williams & Wilkins

Care, P. C. (2017). Segen's Medical Dictionary.(2011). Retrieved October 11 2018 from <http://medical-dictionary.thefreedictionary.com/PMI>

Moore, C. V., Thompson, R. C. A., Jabbar, A., Williams, J., Rasiah, K., Pallant, L., Koehler, A., Graham, C., and Weldhagen, G. F. (2016). Rare Human Infection with Pacific Broad Tapeworm *Adenocephalus pacificus*, Australia. *Emerging Infectious Diseases*, 22(8), 1510–1512. <http://doi.org/10.3201/eid2208.160156>

Index

A

abdominal distension 232, 245, 322
 abdominal rigidity 511
 abdominal tenderness 158
 aberrant infection 202, 228
Acanthamoeba XII, 179, 181 – 182, 189 – 190, 567, 598
 acetaminophen 260
 acid-fast 134, 177, 335, 347, 442, 444, 538, 566, 581 – 582
 Acidosis 562
Adenocephalus pacificus 356, 360, 601
Aedini 467, 494
 aestivo-autumnal 101
 African trypanosomiasis XII, 9, 61, 63 – 65, 67, 69 – 73, 473, 494, 559, 593, 595, 603
 Alexandre Brumpt 76
Allodermanyssus sanguineus 509, 515
 alphavirus 470
 amastigote 25, 32, 34, 78 – 79, 81
Amblyomma 500, 503, 521
 American trypanosomiasis XII, 75 – 77, 79, 81, 83 – 85, 87 – 88, 172, 496, 560, 593 – 595
 amoebiasis 153 – 154, 156 – 162, 164 – 165, 524, 593 – 595
 amoeboma 564
 amphibian 585
 amphotericin 35, 39, 44, 54 – 55, 180, 183, 557 – 559, 568
 anaphylactic reaction 375
 anaplasmosis 456, 504
Ancylostoma XIII, 197, 215, 226, 228, 230, 235 – 237, 240, 312, 314, 327, 369, 570, 598
 aneurysm 79
 angioedema 281, 284, 292 – 293, 308, 322, 573, 576
 angiostrongyliasis 320, 325, 593 – 595
Angiostrongylus 1, 314, 319 – 320, 324 – 326, 579 – 580, 598
 Anisakiasis 321 – 322, 326, 580, 593, 595
Anisakis 314, 321, 325 – 326, 598
Anopheles 98, 105, 119, 128, 240, 268, 457, 459 – 460, 465 – 467, 561
 anthrax 471 – 473
 anthroponotic species 30
 antigenic variation 15, 19, 66, 71

– 72
 antimony 35, 38, 44
Apicomplexa 139, 149, 173, 186
 appendicitis 170 – 171, 203, 205 – 206
 Argyll-Robertson 290, 295
 artemether 117, 412 – 413, 419 – 420, 562
 artemisinin VIII, 117 – 118, 127, 562
 arthralgia 469, 492
 arthritis 17, 178, 292, 301, 307, 316, 505, 574, 576
 ascariasis 206, 212, 220 – 224, 234 – 235, 246, 570, 593 – 595
Ascaris XIII, 1, 5, 7, 197, 207, 212 – 213, 216 – 225, 231, 234, 238, 240, 244, 247, 313, 543, 552, 570, 598
 Ashford 8, 27, 178, 188, 206, 249
 Askanazy 241, 249
 asthenia 169
 asthma 19, 220, 223, 271, 307, 316, 323, 496, 510, 515, 518, 572
 Atkins 64, 70
 atovaquone 127, 147, 175, 186, 565 – 566
 auto-infectious 305
 autoinfection 132, 202, 241, 243 – 244, 249, 307, 362 – 364, 369, 569
 azithromycin 147, 151, 175, 180, 183, 186, 565 – 568
 Azoles 35, 44, 557

B

Babesia XII, 139, 173 – 175, 185 – 186, 499, 501, 521, 539, 565, 598
 Babesiosis 173 – 175, 185 – 186, 501, 505, 513, 593 – 595
 Baerman sedimentation method 528
 Balamuth 182
Balamuthia XII, 179, 182, 189 – 191, 567, 598
 Balantidiasis 167, 169 – 171, 593, 595
Balantidium XII, 167, 169 – 171, 539, 565, 598
 Bancroft 10, 267, 304, 330, 464, 555
 Bartonella 462, 482
 Bartonellosis 462
 Bastian 300, 303
 baylisascariasis 319, 593 – 595
 beaver 11, 17, 313, 323
 beetle 476, 518
 benzimidazole 211, 233 – 234, 238, 379
 benznidazole 82, 88, 560

Bilharz 49, 215, 362, 370, 393, 397, 415, 517
Biomphalaria 395, 402, 420
 Blacklock 280, 286
 bladder fibrosis 409
Blastocystis XII, 97, 182 – 183, 191 – 192, 206, 539, 568, 598
 blepharitis 509
 blindness VIII, 145, 152, 181, 197, 278, 283, 286 – 287, 289, 314, 317, 458, 463, 567, 573, 579
 blister 298, 300 – 301, 500, 512, 518
 bloating 16
 Borovsky 32
Borrelia 1, 174 – 175, 186, 481, 501 – 502, 504 – 505, 513
 botfly 475 – 476
 bradyzoite 141
 Bremser 200, 205
 Brill-Zinsser disease 482
 bronchopulmonary fistula 376
Brugia XIII, 265, 268, 274 – 275, 287, 289, 466, 544 – 545, 572
 bubonic 483, 486
 buffalo 397, 413, 460, 463
 Burkitt's lymphoma 114 – 115, 124

C

Calabar swellings 290, 292 – 293, 295, 308, 573
 cancer 16, 94, 124, 133, 174, 195, 250, 391, 410, 418, 422, 426, 428 – 429, 445
Capillaria XIII, 198, 244, 253, 304 – 305, 307, 309 – 310, 545 – 546, 575
 cardiomyopathy 75, 80 – 84, 86, 88, 292, 573
 Carini 41, 46
 Carrion's disease 460, 462
 cattle 61, 64, 66, 70 – 71, 74, 129, 141, 167, 173, 175, 185, 198, 289, 298, 331, 334, 336 – 337, 341, 374, 385, 393, 397, 413, 430, 435, 456, 475 – 476, 499, 501, 513, 521
 Centipede 519 – 520
 cercaria 5, 391, 403, 406, 408, 433
 Chagas VIII, 50, 52, 57, 75 – 76, 79 – 88, 172, 460, 488 – 490, 496, 598
 chagoma 80
 chancre 64, 67 – 68, 71, 559
 Chandler 228
 Charcot-Leyden crystal 159, 550
 cheilitis 232
 chigger 484, 508 – 509
 chikungunya 1, 456, 470 – 471,

- 494
 cholangiocarcinoma 391–392, 422, 426, 428–429
 cholangitis 221, 376, 426, 429
 cholecystitis 187, 221
 Chrysops 290, 292, 294–295, 460, 471–472, 573, 598
 chylocele 270
 chyluria 270, 274
 ciliated protozoan 167
 cirrhosis 399, 416
 Clonorchis XIV, 422–423, 425, 427–429, 450, 549, 552, 587, 596, 598
 clubbing 210
 Cobbold 265, 267, 274, 290
 cockroach 515, 518–520
 colitis 158, 161–162, 164, 169, 171, 184, 187, 191, 200, 202–203, 205, 207, 210
 copepod 300
 coproculture 247, 251, 571, 577
 coprolites 200, 205, 207
 cougar 254, 261
 Councilman 153, 163
 Coxiella 504
 craterform 32
 crayfish 426, 440, 444
 crepuscular 23, 36, 45
 cribriform 180, 567
 crocodile 254
 crustacean 300–301, 321, 357–358, 425, 441, 443
 crusted scabies 507–508, 514
 Cryptosporidium VIII, XII, 1, 4, 9, 17, 19–21, 129, 131, 133–139, 176, 187–188, 529, 538, 563, 598
 Ctenocephalides 367, 460, 482, 484
 Culicidae 460–461, 464–465, 494
 Culicinae 465–466
 Cunningham 32, 36, 58, 61, 276
 curtisi 98, 102, 122
 cutaneous leishmaniasis XII, 22, 27, 29–31, 33, 35–40, 45–47, 54, 493, 556, 602–603
 Cyclospora VIII, XII, 1, 139, 177–178, 187–189, 538, 566, 598
 cysticercosis 5, 7, 334, 336–337, 339–341, 345–347, 349–353, 385, 388, 582, 593–595
 Cytoisospora 539
- D
 Darwin 76, 85
 delusional parasitosis 458, 493
 Demarquay 267, 274
 Demodex 500, 509, 515
 dengue 1, 456, 458, 467, 469, 471, 494
- Dermacentor 500–501, 503–504
 Despomnier I, III–IX, IX–LXII, 47, 263, 302, 323, 337, 351, 388
 Dientamoeba fragilis XII, 2, 177, 183–184, 187, 191–192, 206, 542, 568
 diethylcarbamazine citrate 267, 284, 292, 308, 603
 diffuse cutaneous leishmaniasis 22, 33, 37, 603
 Digenea 390–391, 443
 Diphyllbothrium XIII, 331, 354–357, 359–361, 383, 547, 552, 583, 598
 Diptera 27–28, 39, 45, 47, 295, 458, 460–461, 494
 Dipylidium caninum XIII, 367, 369, 371, 584
 Dirofilaria immitis XIII, 304, 307, 309, 314, 576
 Dobell 18, 154, 163, 184, 206
 Donovan 52, 57, 196
 dracunculiasis 298, 300–303, 593, 595
 Dracunculus medinensis XIII, 298–303, 574
 Dubini 215, 226, 228, 236, 525
 Dyneshvar 300
 dysentery 5, 153, 158–160, 163, 165, 167, 169–171, 210, 213, 564–565, 569
 dyspareunia 92, 561
 dysphagia 81, 560
- E
 eastern equine 469
 echinococcosis 5, 375–378, 380, 385–388, 593–595, 602
 Echinococcus XIV, 1, 235, 336, 369, 372, 374–380, 385–388, 517, 584–585, 598
 Echinostoma XIV, 448, 453–454, 549, 589, 598
 eflornithine 69, 560
 Egyptian 94–96, 298, 310, 397, 415–416, 436
 Ehrlichia 174, 186
 ehrlichiosis 174, 514
 eicosanoids 6–7, 404, 407
 Ekbom's syndrome 458
 elephantiasis 197, 265, 267, 269–274, 458, 464, 572
 endosymbiont 7, 17, 270, 272, 283, 577
 Entamoeba VIII, XII, 4, 11, 18, 84, 95, 97, 130, 134, 153–155, 157, 159, 161–167, 169, 180, 193–195, 206, 211, 522, 524, 526, 538, 541–542, 564, 598
- enterobiasis 200, 205–206
 Enterobius vermicularis XIII, 200–201, 203, 205–206, 313, 335, 530, 543, 552, 569, 581–582
 eosinopenia 115, 561, 594
 eosinophilia 144, 166, 177, 184, 187, 195, 200, 202–203, 219–220, 232, 245–246, 259, 271, 275, 292, 295, 304–305, 307, 310, 312–313, 316–318, 320–321, 323, 358, 377, 408, 426, 433–435, 441, 448, 566, 570–573, 575–576, 578, 580, 586–590, 594, 605
 eosinophilic 68, 125, 166, 184, 187, 200, 202–205, 219–220, 228, 235, 292, 312, 314, 316, 319–322, 324–326, 407, 426, 436, 445, 579–580
 epiglottis 219, 229, 244
 epilepsy 288, 323, 329, 339, 351
 epimastigote 78, 86
 espundia 41, 43, 46, 604
 exchange transfusion 127, 175, 186, 562, 565–566
 expedited partner therapy 93, 603
- F
 Fantham 61, 64
 Fasciola XIV, 390–391, 425, 430–431, 433–437, 442, 446, 517, 549, 552, 588, 599
 Fascioliasis 430, 433–436, 444, 593–595
 FDG-PET 442, 589, 603
 Fedchenko 300
 female genital schistosomiasis 393, 395, 410, 415, 586, 603
 filaria 240, 267, 274, 276, 281, 286, 288, 292, 294–295, 303, 436, 460
 filarial 7, 10, 152, 269–273, 275–276, 278, 281, 287, 290, 292, 296, 307–308, 310–311, 464, 466, 471, 523, 531, 555, 572, 577
 Finlay 386, 464
 Forbes 300
 Foster 126, 228, 416, 481
 Franciscella tularensis 504
 Frenkel 139, 148
 Fuelleborn 241, 249
 Fujinami 399, 416
- G
 Garnham 99, 122, 225
 Giardia VIII, XII, 2, 4, 9, 11, 13–21, 95, 130, 134, 137, 187, 211, 526, 534, 536, 556, 599
 Giardiasis 11, 15–21, 523–524, 593, 595

- giardavirus 18
 Glisson's capsule 425, 430
 Glossina 61, 70 – 72, 460, 472, 474
 Gnathostoma 314, 320 – 321, 325, 580, 599
 Gnathostomiasis 321, 325, 442, 580, 593 – 595
 granulomatous amoebic encephalitis 181 – 182, 190, 603
 Grassi 48, 98 – 99, 240, 362, 370, 455, 464
 gravlax 359
 Griesinger 397, 416
 ground squirrel 485
 Guillain-Barré syndrome 470, 503
- H**
 Halzoun 433, 435
 hamster 25, 156, 351, 367, 371
 hartebeest 64
 Hartmann 340, 351
 Hawking 267, 274
 Hemiptera 75, 486, 599
 hemozoin 4, 112, 174
 Henderson 139, 148
 hepatitis 486, 496, 502, 504, 513
 hepatobronchial fistula 159
 Hepatosplenomegaly 50, 53, 68, 145, 316 – 317, 407 – 408
 Heterophyes heterophyes XIV, 6, 449, 453, 549, 590
 Heterophyes nocens 449, 454
 Hippocrates 99, 121, 153
 hookworm 7 – 8, 197 – 199, 212, 215, 226, 228 – 239, 244, 246 – 247, 249, 309, 311 – 312, 327, 418, 421, 525, 543, 552, 555, 577 – 578
 Hopkins 20, 126, 163, 289, 302 – 303
 hornet 460, 487, 493
 housefly 472 – 473
 HTLV-1 245, 250
 Hutchinson 139, 360
 hydatid 372, 374 – 380, 385 – 388
 hydrocele 267, 270, 273, 572
 hydrocephalus 139, 144 – 145, 344 – 346, 349, 563, 582
 hydrogenosome 9, 91, 95
 Hymenolepis XIII, 49, 235, 240, 362 – 365, 369 – 371, 518, 547, 552, 583, 599
 hyperinfection 236, 244 – 248, 250 – 251
 hypersplenism 409
 hypnozoite 99, 115, 119, 122 – 123, 225
 hypoattenuating tracts 433
- I**
 intracranial 145, 147, 344, 346, 349, 563
 Iodamoeba bütschlii 194, 541
 iridocyclitis 283
 iritis 283, 573
 ivermectin 152, 211, 233, 248, 251 – 252, 272 – 273, 276, 278, 284 – 285, 287 – 288, 290, 292, 294 – 295, 308, 310 – 311, 313, 319, 321, 471, 480, 495, 508 – 509, 515, 569 – 574, 576 – 578, 580, 591 – 592
 Ixodes 173, 175, 187, 457, 499, 501, 503, 505, 513 – 514, 565
 Ixodidae 187, 498 – 500, 513 – 514
- J**
 Janicki 357, 360
 Japanese 99, 170, 187, 237, 387, 416, 469
 jejunal perforation 334, 337
- K**
 Kala-azar 53 – 59, 148, 196
 karyosome 154
 Katsurada 393, 399, 416, 449
 Kawanishi 399, 416
 keratitis 181 – 182, 190, 281, 283, 287, 567, 573
 Kerbert 438, 444
 Kessel 89, 94, 393
 Kilbourne 10, 173, 464, 499, 521
 kinetoplast 22, 34, 56, 61, 65, 70 – 71
 kinetoplastidae 9, 22, 61
 Kinghorn 64, 70
 Kligler 46
 Kobayashi 425, 428
 Koch's postulates 300
 koilonychia 232
 Krotoski 99, 122 – 123
 Kuchenmeister 303, 333, 336, 340, 351
 Kyasanur Forest disease 504
- L**
 Lafleur 153, 163
 Laveran 98 – 99, 121, 599
 Leeuwenhoek 11, 534, 599
 Leiper 290, 295, 397, 416, 471, 494
 Leishman 52, 57, 148, 196
 Leishmania XII, 2 – 3, 22 – 30, 32 – 41, 43 – 47, 50, 52 – 59, 61, 71, 83, 196, 460, 462, 493 – 494, 557 – 558, 599
 Leishmaniasis XII, 9, 22 – 23, 27 – 31, 33 – 41, 43 – 47, 49 – 51, 53 – 59, 68, 97, 225, 276, 462 – 463, 493, 524, 556 – 558, 593, 602 – 605
 Leishmaniovirus 45
 leopard 282, 286, 288, 438, 573
 Lepidoptera 518, 520
 Leuckart 167, 278, 280, 286, 333, 336, 372, 430, 435
 Linnaeus 200, 207, 216, 218, 298, 339, 354, 357, 360, 367, 430, 435
 littoral vegetation 430, 432
 lizard 325, 585
 Löeffler's syndrome 220, 570
 loiasis 5, 288, 290, 292 – 296, 574, 593 – 595
 Loxoceles 500, 511 – 512
 Lugol's iodine solution 529, 532
 Lutzomyia 22, 27 – 28, 30, 39, 41, 45, 47, 460, 462, 494
- M**
 maggot therapy 475
 Majima 399, 416
 malaria VIII – XVI, 1 – 4, 9, 45, 48, 53, 68, 72, 76, 97 – 99, 101 – 102, 105 – 107, 112 – 128, 170, 172 – 173, 225, 228, 231, 237, 240, 330, 413, 419, 455, 457 – 458, 463 – 466, 470 – 471, 494, 499, 521, 523 – 524, 535, 562, 593 – 595
 Malmsten 167, 171
 Manceaux 139
 mandrill 182
 Manson 10, 162, 267, 274, 280, 286, 290, 294 – 295, 307 – 308, 330, 397, 416, 438, 444, 455, 464, 494
 Mansonella XIII, 294 – 295, 307 – 308, 310 – 311, 460 – 461, 545, 576 – 577, 599
 Marburg 44, 248, 252
 Marchoux 499, 513
 Markus 99, 122
 maxadilan 23 – 24, 27 – 28
 McConnell 422, 428
 McKenzie 290
 mealworm 365
 Meckel's diverticulitis 334, 337
 Mectizan 278, 284 – 286, 288
 Megacolon 79, 81, 87, 560
 megaesophagus 79, 81
 Meisel 129, 135
 Mesocostoides XIV, 382 – 383, 388, 585, 599
 Migone 52
 miltefosine 35, 37, 39, 44, 46 – 47, 55, 59, 180 – 181, 183, 557 – 559, 567 – 568
 Minchin 362
 Miyagawa 399, 416
 Miyairi 399, 416
 mollusk 391

- Mongin 290, 294
 mongoose 321, 438
 Monkey 122, 182, 265
 Morgagni 207, 213
 mucocutaneous leishmaniasis XII,
 22, 29, 37–38, 41, 43, 45–47,
 49, 557, 604

 N
 Naegleria fowleri 179, 189
 nagana 64, 70, 74
 Nakagawa 438, 444
 Nakamura 387, 399, 416
 Nanophyetus salmincola 453–454,
 590
 Naunyn 374, 386
 Necator americanus XIII, 197, 226,
 229–230, 235–238, 311, 525,
 570
 Nicolle 52, 57, 139, 148, 362
 Nishigoi 241, 249
 nodding syndrome 283, 288, 573
 Noguchi 46
 Normand 241, 249
 Norwegian scabies 507

 O
 O'Neill 280, 286, 436
 Ochlerotatus 456, 466–467, 469,
 494
 Oesophagostomum bifurcum XIII,
 307, 309, 311, 577
 Oestridae 461
 Oliver 333, 336, 513
 Onchocerca XIII, 97, 152, 278–
 279, 281, 283, 285–289, 308,
 460, 463, 573, 599
 onchocerciasis 5, 211, 272–274,
 276, 278, 280–290, 294–296,
 308, 412, 415, 463–464, 593,
 595, 602, 604
 Onchocerciasis Control Program
 278, 604
 opisthorchiasis 391–392, 426, 428
 –429, 436
 Opisthorchis XIV, 422–423, 425,
 427–429, 587, 600
 opossum 85
 Orientalis tsutsugamushi 509
 Ortega 177–178, 187–188
 ovoviviparous 265

 P
 Pallas 374, 386
 Panstrongylus megistus 460, 488
 panther 438
 Paragonimiasis 438, 441–445,
 593–595
 Paragonimus XIV, 6, 390–391,
 438–445, 549, 552, 588, 600
 parasitophorous vacuole 2, 4, 78,
 141–142, 144, 174
 pediculosis 477, 495
 Pediculus 460, 477
 pellagra 463, 494
 Pelletier 99
 Pentastomida 519
 periportal fibrosis 409
 Phlebotomus 22, 28, 52, 57, 457,
 460, 462
 Pinkerton 139, 148
 pinworm 17, 200, 202–206, 313,
 530, 569
 Plasmodium cynomolgi 122–123
 Plasmodium knowlesi XII, 105,
 121–122, 126, 561
 Plasmodium malariae XII, 102,
 122, 125, 537, 553, 561
 Plasmodium simium 122
 Plater 331, 336, 357, 360
 post-Kala-azar dermal leishmaniasis
 54, 58, 605
 premunition 117, 126
 primary amoebic meningoencephalitis
 179, 189–190, 604

 Q
 Quincke 153, 162

 R
 rabbit 430
 raccoon 318, 324
 Ransom 218, 223
 Rattus norvegicus 319
 reactivation 53, 81, 87, 123, 145–
 146, 563
 Recrudescence 101, 115
 Reduviidae 486, 488, 490
 reindeer 374, 386
 Renoult 397, 415
 Rhipicephalus 502–504
 rickettsia 453, 481–482, 485, 500,
 502, 504, 509
 Robles 280, 286
 Rockefeller 228, 525
 rodent 50, 123, 364–365, 370,
 482, 485, 505, 509–510
 Roederer 207, 213
 Rudolphi 300, 365, 370
 Ruiz-Tiben 302–303
 Russell 32, 36, 73, 249, 515

 S
 salmon 356, 358–360, 453–454,
 590
 Scabies 495, 506–508, 514–515,
 518, 592–593
 Schaudinn 153–154, 163
 Schistosoma XIV, 49, 330, 352,
 390, 392–393, 397, 400–401,
 407, 415–421, 441, 517, 523,
 528, 548, 552, 586, 600
 Schistosoma haematobium XIV, 49,
 393, 401, 415, 417–420, 517,
 548, 552, 586
 Schistosoma intercalatum XIV, 393,
 415, 417, 586
 Schistosoma japonicum XIV, 393,
 400, 415–416, 418–421, 528,
 548, 552, 586
 Schistosoma mansoni XIV, 330,
 352, 390, 392–393, 397, 400,
 416–420, 528, 548, 552, 586
 Schistosoma mekongi XIV, 393,
 400, 415, 548, 586
 schistosomiasis 192, 226, 285, 338,
 349, 390–393, 395, 397, 399,
 405, 407–421, 435, 523, 586–
 587, 593–595, 603
 Scorpion 500, 512–513, 516
 screwworm 476
 seizure 316, 349
 Sergeant 32, 462
 Shortt 99, 122, 225
 Siebold 49, 331, 336, 362, 370,
 374, 397, 415, 449, 517
 Slavin 129, 135
 spider 500, 510–512, 515–516
 Spirometra mansonioides 330, 360,
 383
 Splendore 139, 148
 splenomegaly 53, 114, 122, 125,
 409, 558, 586
 squirrel 484–485
 Stiles 11, 226, 228, 236, 525
 strawberry cervix 92, 561
 strongyloid enteritis 241
 Strongyloides fuelleborni 249
 Strongyloides procyonis 312, 314
 Strongyloides stercoralis XIII, 159
 –160, 229, 233, 240–241, 243,
 245, 247, 249–252, 305, 362,
 522–523, 526, 528, 544, 571
 strongyloidiasis 187, 241, 245–
 246, 248–252, 324, 593–595
 Suzuki 399, 416
 Swaminath 52, 57

 T
 Tabanidae 295, 460–461, 471
 Taenia XIII–CCLIII, 238, 329,
 331, 333–337, 339–341, 343
 –349, 351–353, 360, 370, 385
 –386, 388–389, 530, 547, 552,
 581, 586, 600
 tarantula 510
 termite 462, 490
 thalassemia 116
 Thomas 64, 67, 127, 330, 430,
 435, 464
 tilapia 450
 Toxocara 313–314, 316–318, 323
 –324, 600

- Toxocariasis 316 – 318, 322 – 324 283, 285, 287 – 288, 292, 295,
 308, 310 – 311, 577
 Toxoplasma XII, 3 – 5, 139 – 143,
 145, 147 – 151, 176, 323, 563,
 600 Wucherer 10, 267, 274, 555
 Wuchereria XIII, 10, 265, 267,
 275 – 276, 281, 311, 464, 466,
 544, 553, 555, 572, 600
 toxoplasmosis 139, 142, 144 – 151,
 225, 524, 593 – 595
 trachoma 278, 285
 Trichinella XIII, 1, 197 – 198, 231, X
 253 – 255, 257 – 264, 280, 286,
 305, 361, 535, 544, 551, 571,
 600 Z
 Trichinellosis 253, 258 – 264, 593 Zenker 254, 263
 – 595
 Trichomonas XII, 2, 9, 11, 89,
 91, 93 – 97, 163, 193, 195, 529,
 536, 540, 561, 600
 trichomoniasis 89, 93 – 96, 195,
 593, 595
 trichuriasis 207, 210 – 213, 235,
 379, 593 – 595
 Trichuris XIII, 7, 159 – 160, 197
 – 198, 207, 209 – 215, 220, 231,
 234, 238, 248, 253, 305, 307,
 417, 543, 552, 569, 600
 tropical pulmonary eosinophilia 271,
 275, 572, 605
 tropical splenomegaly syndrome 114
 Trypanosoma brucei rhodesiense
 XII, 61, 65, 72 – 73, 536, 559
 Trypanosoma evansi 471
 Turkey 21, 372, 434, 463
 typhus 477 – 479, 481 – 482, 485
 – 486, 496, 500, 504, 509, 514
 – 515
 Tyzzer 129, 135

U

Uncinaria stenocephala 312, 314

V

- Vagelos 285
 verruga peruana 462
 Vianna 39, 41, 76, 85
 Virchow 162, 171, 254, 262, 551,
 600
 visceral leishmaniasis XII, 9, 22,
 27, 34, 36 – 37, 39, 44, 47, 50 –
 51, 53, 55 – 59, 276, 558, 605

W

- Wakana disease 229, 232, 236
 walrus 254, 260
 warthog 64
 waterbuck 64
 watercress 391, 430, 588
 Weinland 365, 370
 western equine encephalitis 469
 whipworm 197, 207, 210, 213
 Wilder 313, 323
 Winterbottom 64, 67, 72
 Wolbachia 7, 270, 272, 275, 281,

