Seventh Edition

Parasitic Diseases

Despommier
Griffin
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Hotez
Knirsch

Parasites Without Borders, Inc. NY

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Life Cycles by
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Photographs by
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>400 illustrations in full color

>4,000 references

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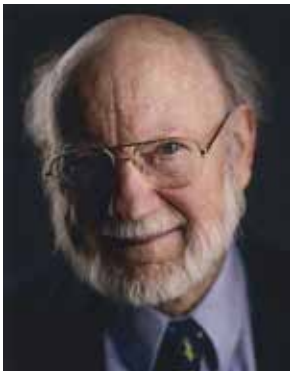
We dedicate this 7th edition to our former President Jimmy Carter and William Campbell. President Carter's leadership and support for the eradication and elimination of neglected tropical diseases was central to alleviating suffering and improving life for the world's most disadvantaged populations. We also acknowledge William Campbell as one of the people responsible for the miracle drug, ivermectin. Campbell was awarded the Nobel Prize in Physiology or Medicine in 2015 for this excellent work.

President Jimmy Carter



William Campbell

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Thanks to all the infectious disease experts (Justin Aaron, Sapha Barkati, Craig Boutlis, Mary Burgess, Matthew Cheng, Lucy Cheng, Elise O'Connell, Priya Kodiyanplakkal, Atul Kothari, Michael Libman, Tim McDonald, Juan Carlos Rico, Jordan Rupp, Keyur Vyas, Amy Murillo and Johnnie Yates) who volunteered their time and expertise to review this textbook prior to its publication. Appreciation to our entomology experts Jonathan Larson and Amy Murillo for many suggestions and additions to the Arthropod sections. Thank you to all our donors who continue to make it possible for us to get this book into the hands of those who need it the most.

Preface

The accumulation of new knowledge over the last several years has provided the impetus for this, the 7th edition of Parasitic Diseases. By integrating all the salient information contained in over 1000 new references with the current descriptions of each pathogen, we continue the never-ending process of evaluating, revising, and improving our understanding of these parasitic organisms. New features to the 7th edition include a clinical summary section, additional life cycle diagrams and a pronouncer's guide to parasite names. Consideration of the biographies of those notable contributors to the field of parasitology has also been extended. Parasites Without Borders continues to make available, free of charge, the PDF version of Parasitic Diseases in both English and Spanish editions.

The number of eukaryotic parasites whose entire genomes are available since completion of The Human Genome Project in 2003, continues to grow and these are highlighted in our 7th edition. Genomic datasets hold great promise for the development of new vaccines, drugs, and control programs based on identifying unique molecular pathways essential to each pathogen. These on-going projects serve as a living testament to the perseverance of a small group of creative, highly trained researchers, whose discipline and dedication helps stem the spread of these life-threatening entities.

Progress in rapid identification of parasites in the diagnostic laboratory by targeting their genomic signatures in biological specimens has dramatically improved and simplified diagnosis and is now the preferred approach in most hospitals throughout the developed world. While molecular-based testing has replaced the microscope as the method of identification in the vast majority of laboratories, in the less developed world traditional approaches to the identification of parasites remain the only technology available. Hence why we still include a diagnostic atlas in our text.

Recent advances have also improved our understanding as to the molecular mechanisms underlying the subversion of our signaling pathways by some parasites, mainly the *Leishmania* spp. and *Toxoplasma gondii*. Thus, we now have a much clearer picture of how they survive so long within us without incurring harm to themselves and will undoubtedly inspire the development of the next generation of more effective chemotherapeutic agents that take advantage of these data. Unraveling the biochemical complexities of how some parasites manage to survive for long periods within the human host has also provided clues in the treatment of illnesses unrelated to the parasites themselves. "Swords into plowshares" molecular strategies, are providing "parapharmaceuticals" for coronary artery disease, stroke, and autoimmune disorders. Clinical trials evaluating the prophylactic use of hookworm-based peptides and eicosanoids that block host clotting, host platelet aggregation, and host inflammation are underway at the time of publishing this 7th edition.

An explosion of new compounds due to advances in drug discovery and design using high-throughput screening algorithms, cryo-electron microscopy, and virtual reality visualization technologies now await testing. These approaches have already provided the clinician with a new generation of drugs, many with less harmful side-effects than the ones they replaced. Controlling parasite populations at the community level is an anticipated consequence of such

developments, without the risk of harming the very ones we wish to help.

The remarkable success of programs such as those targeting river blindness, which has been controlled in many countries in West Africa, demonstrates that when political will and strong social support combine, we can successfully limit the spread of parasites. By targeting and managing drug use in such control strategies, it is possible to eradicate parasites by disrupting their life cycles in a localized manner. Almost all regions of Africa have brought dracunculiasis under control. Similarly, the southern cone initiative of South America has succeeded in dramatically reducing cases of Chagas disease.

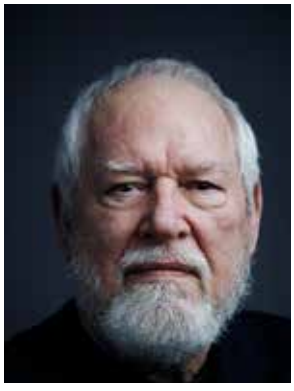
Primarily driven by the U.S. President's Malaria Initiative and the Global Fund, the number of children who die from malaria has fallen by more than 50% since 2000, saving over 6 million lives. Despite all this progress, there are still high rates of morbidity and mortality throughout the tropics due to some of the most commonly occurring parasitic infections, especially malaria. While there are no new classes of drugs for treating resistant malaria, artemisinin-derivatives continue to be effective in reducing the mortality of the world's most devastating infectious disease wherever that chemotherapeutic agent is available. For millennia, worm infections have exacted their toll on humanity, with children as their primary victims. Until basic sanitation is implemented this will, regrettably, be the norm for most underdeveloped countries and impoverished communities.

Due to the political instability of vast regions of Africa and the Middle East, the re-emergence of many infectious diseases, including leishmaniasis and African trypanosomiasis, has become a significant problem. This is primarily due to environmental destruction, abandonment of control programs, and forced migration of tens of thousands of individuals from regions that were relatively safe, to places that no one should have to occupy, no matter how short the duration. These seemingly intractable situations require more than vaccines and drugs to affect a cure. Social stability, equity, economic development, and long-term planning are the "drugs of choice".

The impact of HIV/AIDS in resource-constrained areas continues to reduce life expectancy significantly. While the immunosuppressive effects caused by this disease and the impact on other parasitic diseases is still poorly understood. Such effects require careful monitoring. As access to antiretroviral therapy improves due to the Global Fund and other non-governmental entities, new clinical syndromes are likely to emerge due to parasites behaving differently in hosts with an ever-changing immune status.

Diarrheal diseases caused by a variety of infectious agents, including *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, and *Cyclospora cayetanensis* round out the list of miseries that those living in poverty in the less developed world endure. Where feces and urine serve as the best source of fertilizer, education as to how to apply basic sanitation to render them into safe manure, remains high on the list priorities.

Ultimately, it is through the education of students, clinicians, and parasitologists and vigilance of commitment that we can hope to improve the lives of hundreds of millions of less fortunate individuals by helping them live longer and more productive lives. The 7th edition of Parasitic Diseases is dedicated to this premise. We invite you to join us in this global effort.



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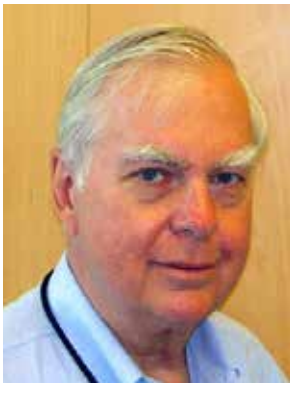
Daniel O. Griffin



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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 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

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 encompass subsets of organisms within the protozoan and helminth (parasitic worm) groups. In addition, medically important arthropods have been included in discussions of eukaryotic parasites, since so many of these pathogens are transmitted to humans by arthropod vectors. Besides, some medically relevant arthropods cause disease on their own.

From a biological perspective, a phylogenetic presentation of eukaryotic parasitic organisms would undoubtedly satisfy those specialists who strictly adhere to the zoological literature, while most medical students and practicing clinicians would have little or no use for this information. The physician is more inclined to group them according to their syndromes, if they were to classify them at all. We have settled upon a compromise, in which these organisms are encountered by the reader in a somewhat biologically correct order, together with an outline of their classification and clinical presentations. Nonetheless, it is in some sense intellectually satisfying to review parasitic organisms with a semblance of evolutionary precision, allowing each student to learn about them in a sequence that most experts in the field of parasitology have agreed upon, going from the single-cell parasites to the worms and beyond. We, therefore, present protozoans first, followed by the helminths, and finally round out the synopsis with medically relevant arthropods.

The following sections are organized in such a way as 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. Thus, 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. mechanisms of survival (i.e., virulence factors), and 5. mechanisms of pathogenesis.

The last half of the twentieth century has been a remarkable one for the community-based control of pathogenic organisms. New vaccines and antibiotics have also helped reduce the incidence of numerous pathogenic organisms. At the same time, it has also heralded the emergence and re-emergence of a wide spectrum of infectious agents: viruses (e.g., SARS, HIV, monkey pox, 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 been able to take advantage.

It is difficult for even the most attentive student, to truly comprehend the prevalence of, and suffering caused, by parasites. This is especially true when hearing a very large number, as is the case for *Ascaris lumbricoides*, which infects hundreds of millions of people around the world. So, when one hears for the first time that 100s of millions of people are infected with malaria each year, and over ½ a million children per year die, in Africa alone, from this infection, these facts seem somehow remote, even abstract. Yet, when a single child suffering from the cerebral form of this disease-causing entity is admitted into a modern hospital in critical condition, and, regardless of treatment, that young person dies, the health care community

of that institution is put into collective shock. If the death occurred at a teaching hospital, a grand rounds is the usual outcome, perhaps motivated by some vague sense of guilt, in an attempt to see if anything could have been done to spare that life. Unfortunately, the most lethal species of malaria, *Plasmodium falciparum*, is evolving more and more resistance to the medications in our arsenal.

Parasitic Protozoa

What is a protozoan? Which ones cause disease? How do those that are parasitic differ from their free-living counterparts? What are the pathogenic mechanism(s) by which they cause disease? There are over 200,000 named species of single-celled organisms that fall under the category protozoa, while many more, no doubt, await discovery. Only some small fraction of these is parasitic for the human host, yet some can cause great harm (e.g., malaria), especially when they are encountered for the first time.

Protozoans are single-cell organisms inside of which usually resides one membrane-bound nucleus, with a few exceptions, such as *Giardia lamblia* and *Dientamoeba fragilis*. Most protozoa have one type of organelle that aids in their movement (e.g., flagella, undulating membrane, cilia). Metabolic pathways vary from group to group, with both anaerobic and aerobic energy metabolisms being represented among the parasites to be discussed. In the case of 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 give off substances 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. Some attention to both the biochemical and molecular biological findings for a given organism will be presented whenever they have relevance to the understanding of the mechanisms of pathogenesis or parasite survival strategies.

Mechanisms of Entry

Protozoans gain entry into their host in one of several pathways: oral, sexual, 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 important aspects in that regard.

Many species of 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 enabling them to lie dormant in the environment for long periods of time, 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. Certain amoeba may 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 species of malaria (*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 blood

stream or interstitial tissue fluids where they proceed to undergo complex developmental life cycles culminating in numerous cycles of asexual division once they achieve their essential niche.

A more complex strategy is employed by *Trypanosoma cruzi*, an organism transmitted by a large hemipteran with ferocious looking biting mouthparts. In this instance, the organisms are excreted along with the fecal exudate at the time of the second blood feeding. Humans become infected unknowingly, by rubbing the organisms into the bite wound or into a mucous membrane after the insect 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 the anatomical, physiological, and biochemical features of that site. To gain some measure of the difficulties associated with attempting to describe the essential niche, be it that of a parasite or any other organism, let us consider the intracellular milieu of the normal red blood cell. This site represents one of the best studied of all intracellular environments. Yet for the most part, we still do not understand precisely how that anucleate cell's membranes interact with vascular endothelial cells when the cell traverses the capillary and exchanges gases with the surrounding tissues. To make matters worse, a red blood cell infected with *P. falciparum* behaves quite differently from that of a normal one, failing to deform as it enters the capillary bed. This single aspect of the infection has serious pathological consequences for the host, as will be detailed in the section dealing with the clinical aspects of malaria.

The internal molecular environment of the infected red cell must be considered as a "hybrid," consisting of both host and parasite elements. Proteins, produced by the developing merozoite, locate to the cytoplasm of the host cell, and some even integrate at the red cell membrane surface, forming complexes with host structural proteins such as spectrin and glycophoran. Others remain in the general region of the red cell cytoplasm. Over the entire period of the developmental cycle of the parasite, new proteins are produced that locate to specific regions of an ever-changing host cell environment. The infected red cell represents a very dynamic situation; even with the most sophisticated instrumentation, it has been impossible to fully appreciate the setting in which this important pathogen lives out its life.

Finally, no two species of *Plasmodium* behave the same in their erythrocytic niche, due largely to dramatic genetic differences between the major species infecting humans. Hence, it is likely that we will 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 secret life.

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

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 are capable of infecting cells that under most circumstances would serve to protect us

from these kinds of organisms. The macrophage is a permissive host cell for *T. gondii* and for all species of *Leishmania*. In these infections, the very cell-type we depend upon for innate protection against invaders turns out to be the culprit, aiding in their dispersal throughout the body.

Division and Reproduction

Multiplication within the human host is the rule for protozoans, in contrast to most helminth species, in which infection usually results in a single adult parasite. The definitive host is the one harboring the sexual stages or the adult stages of a given parasite. Hence, the human is not the definitive host for a wide range of protozoan infections, including the *Plasmodium* spp. and *T. gondii*. Female anopheline mosquitoes are the definitive hosts for all malaria species infecting humans, while the domestic cat and other Felidae are the permissive host for the sexual stages of *T. gondii*. Humans are the definitive host for *Cryptosporidium parvum*. It should be emphasized, however, 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. During the height of the infection, they place ever-increasing demands upon their essential niches. The mechanisms by which protozoa divide asexually are numerous, with binary fission being the most common. Malarial parasites reproduce within the red cell by a process called 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, crystals of hemozoin, in the now empty red cell stroma.

Mechanisms of Survival

Each species of parasite has been selected for life within the human host 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 that are incapable of protective immune responses. For example, the African trypanosomes produce "smoke screens" of surface antigens whose sole purpose seems to be to keep the immune system busy, while a small select population changes its protein coat to a different antigenic variant, thus temporarily escaping the host's immune surveillance system. Certain stages of the malaria 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 an 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* actually escapes the parasitophorous vacuole into naked cytoplasm, avoiding the ravages of lysosomal enzyme activity. 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 inflicted by a given parasite is related to the location of their essential niche, the metabolic requirements of the parasite, and their population density throughout the infection. Energy is derived from the host, placing a burden on infected

hosts for providing this essential ingredient. The penchant of the parasite for killing the cell it invades or eroding away 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 results in the release of toxic waste products (hemozoin) that elicit fever. *Entamoeba histolytica*, as its name implies, attaches to, then ingests living cells. It then digests them, using acid hydrolases to do so, and in the process induces bloody diarrhea (dysentery).

Infection with *T. gondii* results in lymphedema and fever due to the death of large numbers of host cells throughout the body. The molecular basis for these pathological effects will be discussed in detail at the appropriate time. Suffice it to state here that we do not know any parasite's modus operandi completely, and scientific endeavor will undoubtedly continue to provide surprises and revelations in the near future.

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 two are endoparasitic to humans. Both the Nematoda and Platyhelminthes have many free-living species as well. A general description of each major group precedes each section. What follows is a general description of their biology.

Mechanisms of Entry

Helminths have evolved multiple strategies for entering the host and establishing infection. Among the nematodes, infection is usually established by exposure to an environmentally resistant stage.

For many of the common intestinal nematodes such as *A. lumbricoides* or *Trichuris trichiura*, this occurs via the ingestion of embryonated eggs in the soil, or on fecal-contaminated fruits and vegetables. 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 the soil, enter the host. 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 infection (copepods).

Trematodes spend a portion of their life cycle in a wide variety of 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. The *Schistosome* cercariae can penetrate through the hair follicle, the invaginated epidermal layer that forms the hair shaft, due to the thinner epidermis.

Cestodes are acquired via the oral route, regardless of the stage that ends up causing the infection. Most adult tapeworm infections of humans result from the ingestion of 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, in order to arrive there, the infective larvae frequently must first pass through the skin and lymphatics before spending time in the bloodstream and lungs before migrating via the esophagus back to the gut. Similarly, *Ascaris* eggs hatch in the intestine before the emerging larval stage enters the portal circulation; the larvae enter liver and lungs prior to re-entry into the gut. As adults, helminths have been recovered from almost every organ including liver, lungs, lymphatics, bloodstream, muscle, skin, subcutaneous tissues, and brain.

Many species of parasitic helminths (nematodes, cestodes, and trematodes) 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 intestine, with each species occupying a different portion of the gut track. Symptoms arising from heavy infection with a given helminth are typically associated with a particular region of the GI tract.

Reproduction

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

In the cestodes, the situation is somewhat different as each proglottid segment of the adult cestode tapeworm is hermaphroditic. Because there is usually only one adult worm 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 then disintegrate releasing their eggs into the fecal mass for export. Juvenile tapeworm infections remain as such and produce no diagnostic stage. These infections present real problems for the clinician seeking a definitive diagnosis for their patient.

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, as 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 in order to ensure that their eggs leave the human host. For instance, schistosome eggs are deposited against the inside wall of a blood vessel. These eggs are equipped with sharp spines and a battery of lytic enzymes that allow them to traverse the vessel endothelium and gut wall. The eggs traverse through the serosal surface of either the intestine or bladder (depending upon the species), before entering the muscularis layer and then the lumen. Adult *Schistosomes* and *Paragonimus* that locate to ectopic sites (e.g., nervous system) produce 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 habitats which most of us would 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, a place where one might expect to encounter the constant bombardment of the immune system's slings and arrows of antibody molecules and leukocytes of various types. Yet, there the worms can thrive for up to twenty years in that niche.

The molecular basis by which this happens is not known, although a number of immune evasion and immunological masking mechanisms have been described. Important for helminth survival is their unique array 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 themselves have proven to be useful as new potential therapeutic agents for human coronary artery disease, stroke and autoimmune disorders. *T. 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, which, through research and development in the form of millions of years of evolutionary selection, now produce a wide array of pharmacologically active compounds, which we may eventually find useful, as well.

Mechanisms of Pathogenesis

Helminths injure their human host both through mechanical and chemical mechanisms. Large helminths, such as *A. lumbricoides*, can cause physical obstruction of the intestine, or exert damage when they migrate into the biliary tree. As already 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. There is some evidence to support the role of helminths as co-pathogens that promote susceptibility to HIV infection and AIDS. In many cases, some of the most important mechanisms of pathogenesis are still not known. Heavy infection with some intestinal nematodes (e.g., hookworm) are considered to be the major cause of stunted growth during childhood as well as inducing impaired cognitive behavior and intellectual development. While intuitively we might suspect that parasite-induced malnutrition plays an important role in this process, the true basis by which these processes occur is not known.

Host-mediated immunopathology accounts for a large measure of the damage that occurs 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*, may be responsible for most of the pathological consequences of the infection. Brain parenchymal inflammation and seizures in cysticercosis are well documented.

The genomes of many of these important pathogens of humans are now available, so new approaches to the clinical management of patients suffering from them are sure to emerge from the laboratory and find their way to the bedside. At least that is the hoped-for outcome of such research.

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 35,000) includes more than 10,000 that have adapted for life as parasites. Regardless of their lifestyle, all protozoans are eukaryotic single-cell organisms. Free-living species occupy every conceivable ecological niche, including marine trenches, rainforests, artesian and thermal springs, salt lakes, ice flows, glaciers, and many others, while parasitic protozoans infect a wide spectrum of vertebrate and invertebrate life.

Unlike the great majority of parasitic helminth species, protozoan parasites are able to replicate within a given host, often resulting in hundreds of thousands of new individuals within just a few days of initial infection. This single feature of their life cycle frequently has grave consequences for the host.

Parasitic protozoans have played a major role in the evolution of the human species, mainly due to lethal consequences of infection, or limiting where people can live by adversely affecting their livestock. These very same selection pressures continue to play out in many parts of the world today. For example, malaria in all its forms, African trypanosomiasis, and visceral leishmaniasis infect millions of people and are responsible for untold numbers of deaths and debilitating chronic illnesses. Many others cause less severe disease (e.g., chronic diarrhea) that nonetheless results in lost time at work and school and loss of recreational activities we deem vital to living enriched, healthy, disease-free lives. 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. There are no effective vaccines for the control of any protozoan infection in humans.

While the biology of parasitic protozoa varies widely from group to group, these organisms share many common features. A unit membrane that functions in a similar fashion to all other eukaryotic cells binds them. 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 protozoans can divide asexually, usually by binary fission. In some instances, the process is more complex, and includes multiple nuclear divisions followed by cytokinesis. Those capable of sexual reproduction do so within the definitive host, resulting in the formation of a zygote.

In addition, 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.

Some species have evolved elaborate surface coats consisting of materials derived from the host or secreted by the parasite that offer some protection from host immune responses, thereby extending their life within a given individual and resulting in great damage to the host as well.

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, the details of protective host mechanisms that counter the invasion process have been described, giving hope for the development of 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 useful and practical information specific to the diagnosis, treatment, and management of 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\\lăm'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 respires as an anaerobe, and lives in the small intestine. Other protozoa sharing this metabolic strategy include *Entamoeba histolytica* and *Trichomonas vaginalis*. The genus *Giardia* is divided into eight genetic groups, with groups A and B infecting humans.[2] *G. lamblia* produces a cyst stage that is environmentally resistant.[3]

Infection is via the fecal-oral route, typically through exposure to contaminated drinking water.[4] *G. lamblia* has a worldwide distribution, and is endemic in many regions.[5] 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.[6-11] *G. lamblia* is a common infection in humans and domestic animals in the United States[12]. It is likely that many infected individuals remain undiagnosed, harboring *G. lamblia* without obvious symptoms.[13]

Beavers are major reservoir hosts and are often responsible for contaminating public drinking water. This is why infection with *G. lamblia* is known in many parts of the United States by the common name of "*beaver fever*".[14, 15] *Giardia* is the subject of much intensive research, including the complete sequencing of its genome.[16] 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.[17, 18] Although an excellent review of the biology of *G. lamblia* was published by Adam in 2001, much has been added to the field since this comprehensive report.[19]

Historical Information

Antonie van Leeuwenhoek (\LAY-vun-HOOK\, \lā-vən-ˌhůk\), the famous Dutch microscopist, in a letter written to Robert Hooke (\HOOK\, \hůk\) 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.*"[20]

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.[21] His elegant scientific drawings remain impressive, even in today's world of sophisticated, technologically advanced light microscopy. In 1921, C.E. Simon completed the description of its morphology.[22]

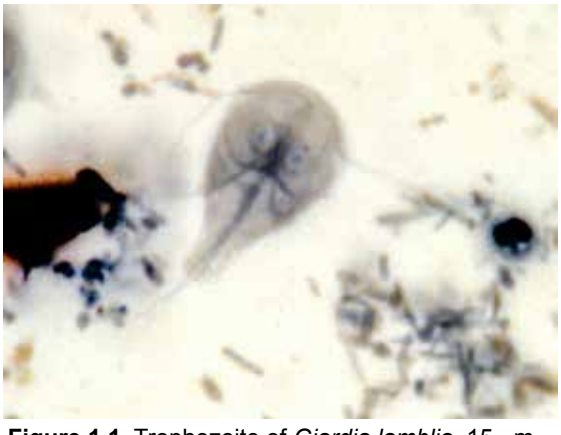

Figure 1.1. Trophozoite of *Giardia lamblia*. 15 μm.

Giardia lamblia

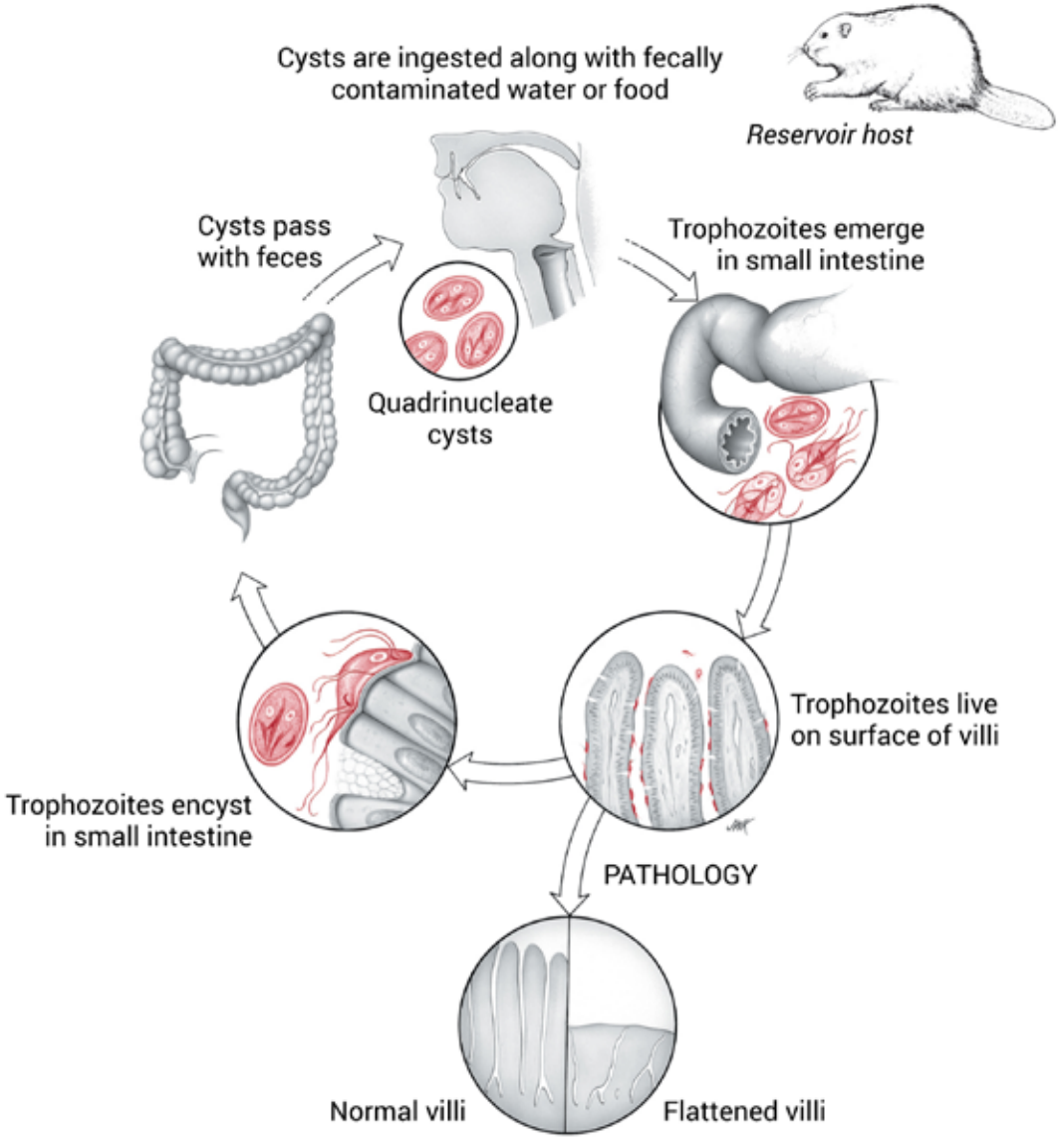

Figure 1.2. Cyst of *G. lamblia*. Two nuclei can be seen. 13 μm.

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.[23] In addition, it contains two rigid structures, called median bodies, which are now known to be part of the complex and unique cytoskeleton.[24] *G. lamblia* has no mitochondria, peroxisomes, hydrogenosomes, or related subcellular organelles that might be associated with energy metabolism, but does appear to use a homolog of the mitochondrial-like glycerol-3-phosphate dehydrogenase (GPDH) that is involved in glycolysis.[25] Some strains of the parasite carry double-stranded RNA viruses, known as G*iardiaviruses*, whose impact on virulence is still being explored.[26, 27] These viruses have facilitated the expression of foreign genes in *Giardia*, serving as shuttle vectors.[28]

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.[29] The latter are members of the class III, low affinity, calcium binding annexins.[30] The disc structure has been investigated using cryoelectron microscopy.[31]

Infection begins with ingestion of the quadrinucleate cyst, which must then excyst in response to a complex sequence of environmental cues received by the parasite.[32] 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 then pancreatic enzymes.[33, 34] Excystation may be inhibited by ethanol and isopropanol-containing hand sanitizers.[35]

Each cyst produces two bi-nucleate trophozoites that then 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.[36] 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 full nutritional needs of the trophozoite have yet to be fully determined, but some of its biochemical energy pathways are known.[37, 38] Glucose and arginine appear to be its major sources of energy, and it may access a portion of its need for them through the breakdown

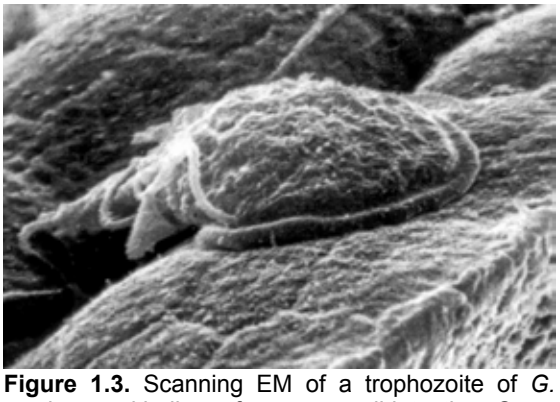

Figure 1.3. Scanning EM of a trophozoite of *G. muris* on epithelium of mouse small intestine. Courtesy R. Owen.

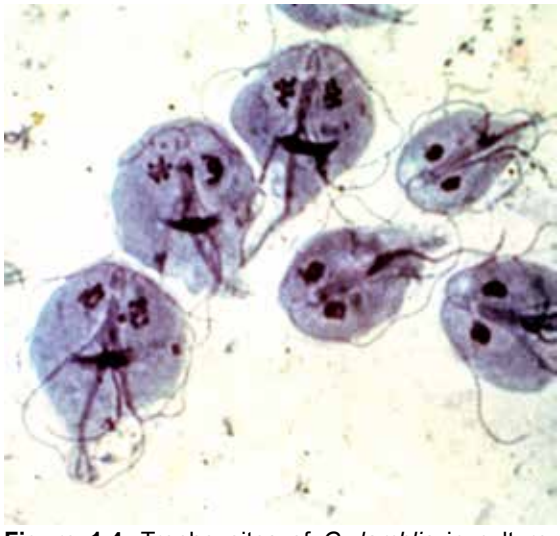

Figure 1.4. Trophozoites of *G. lamblia* in culture. Courtesy D. Lindmark.

of mucus.[39, 40] *Giardia* is unable to synthesize nucleic acid bases *de novo* and consequently employs salvage pathways.[41] Lipids are absorbed directly, likely facilitated by bile and bile salts, and perhaps by endocytosis of lipoproteins.[42, 43]

G. lamblia is not considered an invasive or tissue parasite, but 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.[44] To exit the host and survive, trophozoites must encyst, and pass into the large intestine. Bile salts seem to be involved in triggering this process.[3, 45, 46] Encystation *in vitro* is achieved by exposing the trophozoite stage to bile and elevated pH, possibly stimulating the parasite to sequester cholesterol.[47] Trophozoites take up and release conjugated bile salts.[46, 48] These conditions may be necessary for its survival in its essential niche (Fig. 1.3). A transglutaminase may also be required for encystment.[49]

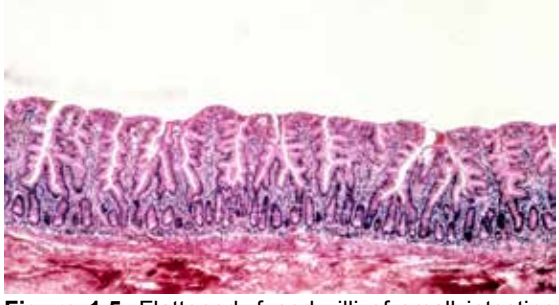

Figure 1.5. Flattened, fused villi of small intestine from a patient suffering from malabsorption syndrome due to *G. lamblia*.

Encysted parasites can endure for long periods of time outside the host if they remain moist and the temperature is not elevated.[50] 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 of time at low temperatures.[51] Freezing, boiling, UV exposure or desiccation can destroy cysts.[52] 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.[53] Protective immunity appears to be directed against both surface antigens and antigens that are secreted.[54, 55]

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.[56, 57] Despite the fact that there are numerous related species of *Giardia*, and that they 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.[19] It appears that the diarrhea generated by *G. lamblia* is, in part, due to augmentation of peristalsis and alteration of host cell tight junctions.[58, 59]

Infection with *G. lamblia* induces numerous cellular and humoral responses, some of

which are protective in nature.[60-62] Secretory IgA is particularly important to the control of the infection.[63] Physiological changes, such as flattening of the villi, experienced during symptomatic infection could relate to these host-based responses, and might even be induced by mechanisms related to allergies, such as those observed in gluten-sensitive individuals.[61, 62]

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.[64] Switching of cysteine-rich variant surface proteins also occurs when the parasite is about to excyst, assisting the parasite to evade immune elimination.[54, 63, 65, 66] Severe combined immune deficiency mice do not induce variant surface protein switching, an indication that the overall process is under the control of B cell-mediated host responses. Shuttle viral systems for transfecting *G. lamblia* have been developed.[67] 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.[68] Nonspecific 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*.[69-71] NO released luminally by intestinal epithelial cells in response to infection, inhibits parasite growth and differentiation, although *Giardia* might be able to disarm this potential defense mechanism by competitively consuming the arginine needed by the host cells for NO synthesis.[72] In summary, the duration and severity of infection depends upon both immune and nonimmune host defenses, as well as the parasite's ability to evade them.

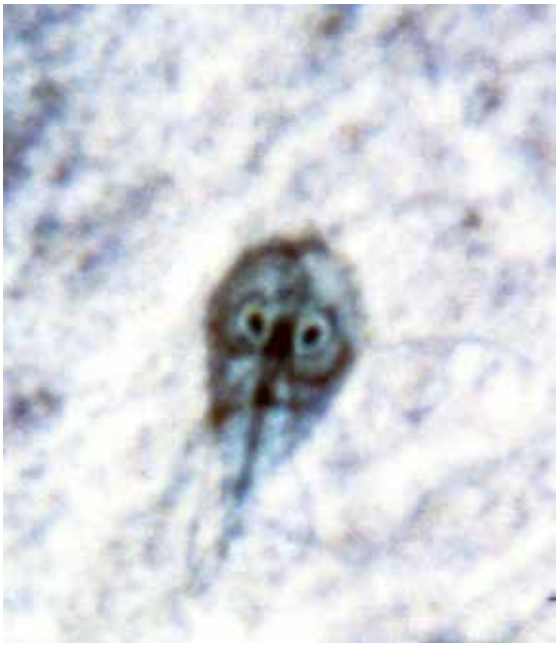

Figure 1.6a. *G. lamblia* trophozoite in stool sample.

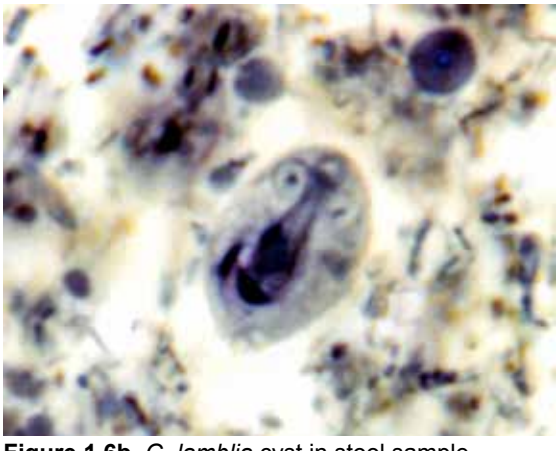

Figure 1.6b. *G. lamblia* cyst in stool sample.

Clinical Disease

It is estimated that a large portion of those who encounter *G. lamblia* and become infected fail to progress to a state of ill health.[73, 74] 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.[40, 75] The acute diarrhea of giardiasis is classically described as foul-smelling with

flatulence, nausea, weight loss and abdominal cramps with bloating.[76] A minority of patients may describe systemic symptoms such as fever.[76] Untreated, this type of diarrhea may last weeks or months, although it usually varies in intensity. Affected children often fail to thrive.[77] Chronic infections are characterized by steatorrhea accompanied by malabsorption syndrome associated with rapid, substantial weight loss, general debility, and consequent fatigue.[40] In addition, some people may complain of epigastric discomfort, anorexia, and even pain.

Certain patient groups are at greater risk for acquiring giardiasis and for 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.[11, 78-80]

Diagnosis (see Clinical Appendix)

The diagnosis of giardiasis has changed dramatically with the introduction of newer diagnostic techniques. Definitive diagnosis still depends upon direct, microscopic observation of trophozoites (Fig. 1.6a) or cysts (Fig. 1.6b) in concentrated stained stool.[81] Due to the challenges inherent in obtaining such specimens and the limited number of skilled laboratory personnel needed to examine them, antigen-capture ELISA was introduced.[82] 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.[83, 84] The string test, which involved swallowing a gelatin capsule attached to the end of a long string, is now relegated to a place in history as newer better-tolerated diagnostic techniques are becoming available. [85] 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.[86-88]

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.[89] The nitroimidazoles, metronidazole, and tinidazole, are the primary drugs used for treatment.[89, 90] 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 g with high efficacy. Another preferred drug option is nitazoxanide 500 mg by mouth twice a day for three days. Recommended doses of these medications are weight based in children. Alternative antimicrobials include paromomycin (in pregnancy), furazolidone, quinacrine, and albendazole.[89]

Recurrence or persistence of symptoms should be evaluated carefully, as persisting malabsorption and lactose intolerance can last for weeks to months following infection.[91] Prior to retreatment it is recommended that one re-evaluate the patient and confirm the presence of infection.[92] 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.[93-96] In some refractory cases, combination antimicrobial therapy may be necessary.[97]

Prevention and Control

G. lamblia is primarily a water-borne infection, although food handlers and infected children in daycare centers no doubt play important roles in transmission.[4, 98] 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 standards of hygiene in daycare centers and mental institutions. A murine model for a protective *Giardia* vaccine exists; however, efforts to develop clinical candidate vaccines, including work on canine vaccines, are hampered by the lack of a well-articulated medical need for commitment of new resources in the setting of many effective therapeutic options.[99]

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2. Introduction to the *Leishmania* species

Pronunciation: \lēsh-ˈma-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]

Leishmania spp. are primarily zoonotic in nature, infecting a wide range of vertebrates throughout the tropical and subtropical world.[4, 5] All *Leishmania* spp. possess a well-characterized kinetoplast, a specialized organelle unique to the kinetoplastidae.[6] 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.[7] Species of *Leishmania* share many similarities in terms of genetics, mode of transmission, biochemistry, molecular biology, immunobiology, and susceptibility to drugs. Divergence is greatest between the cutaneous and visceralizing species of *Leishmania*.

Leishmania spp. are known to cause several different disease manifestations including visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), diffuse cutaneous leishmaniasis (DCL), and less commonly leishmaniasis recidivans (LR).[8] The exact number of humans suffering from leishmaniasis is unknown, but it is estimated that there are 0.2–0.4 million cases of VL and 0.7–1.2 million cases of CL each year.[9] More than 350 million people live within an area of transmission.[9] Leishmaniasis occurs in 88 countries located in Southern Europe, Africa, Asia, South Asia, and South and Central America.[9] The subgenus *Leishmania* is distributed throughout the Old and the New World, whereas the subgenus *Viannia* is only found in the New World. 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 chagasi, 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*.

Figure 2.1. Sand fly taking a blood meal.

The clinical conditions caused by *Leishmania* spp. vary greatly, depending upon the species of *Leishmania* and the immune status of the host. Disease can present as cutaneous lesions that resolve over time, or as systemic disease of the reticuloendothelial system, which often results in death of the host 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, with the tacit assumption that 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.[10]

There are no commercially available vaccines yet, but infection with many of the species of *Leishmania* results in permanent immunity to reinfection with the same species.[11] Perhaps data derived from the genome project will hasten the development of an effective, cheap, easy-to-administer vaccine against the most dangerous forms of leishmaniasis. The development of a vaccine for both animals and humans is an active area of research. [12]

Life Cycle

The sand fly

Infection of the sand fly begins when the insect obtains blood from an infected mammal. Only female sand flies feed on blood.[13] This is a requirement for egg production. The fly injects saliva containing numerous well-characterized bioactive components, many of which are peptides or proteins.[14, 15] One such protein, maxadilan (a potent vasodilator), is a 7 kDa peptide believed essential to the taking of a blood meal by the fly.[16] Maxadilan's primary mode of action is to reduce intracellular calcium in the host at the site of the bite wound through a cAMP-dependent mechanism, causing arterial dilation.[17, 18] Blood can then easily 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.[17] During feeding, infected sand flies become maximally filled with blood and cannot regurgitate the excess, due to the inhibition of the emptying reflex by a parasite-specific peptide that interacts with myosin to prevent contraction of stomach muscle.[19] This enhances the chances for the sand fly to become infected and to remain so throughout the period that the parasite needs (1–2 weeks) in order 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 1 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.[20] 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.[21, 22] The release of infectious stage organisms, a necessary final step in their development, is mediated by arabinosyl capping of lipophosphoglycan scGal residues upon differentiation to the metacyclic stage.[23] The leptomonad stage locates to the anterior region of the gut and 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.[24, 25] Following injection of the metacyclic promastigote stage there is a rapid infiltration of neutrophils into the skin.[26] Several types of tissue-resident phagocytic cells quickly take up the promastigotes.[27] Maxadilan, produced

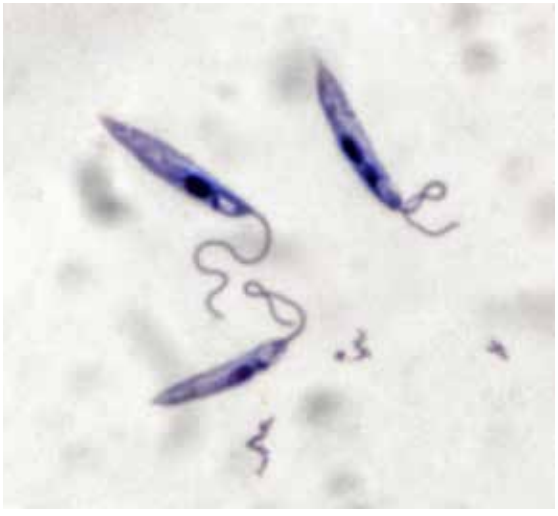

Figure 2.2. Promastigotes of *Leishmania* spp., as seen in culture.

by the parasite, induces negative 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.[28] This all leads to a down-regulation of Th1-type cytokines and a shift to a Th2 response.[29]

The promastigotes deposited in the extracellular matrix at the site of the bite adhere there, aided by lipophosphoglycan and a surface membrane laminin receptor protein on their surface.[30] The promastigotes induce the production of antibodies and become opsonized. As a result, the complement C3 protein attaches to the parasite cell surface.[31] The promastigotes are then able to attach to red cells or platelets and become engulfed by dendritic cells or macrophages (Fig. 2.3).[32] Many would-be pathogens are unable to 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.[33 34] Infected phagocytes display abnormal maturation of the phagolysosome due to lipophosphoglycan's interference with F-actin, an essential component of the process of fusion of lysosomes with the phagocytic vacuole.[35] This lack of fusion enables the parasite to evade digestion.

It is at this point in the life cycle that differences between species of *Leishmania* become apparent. Those that cause only cutaneous lesions remain at the site throughout the infection, while those that cause visceral or mucocutaneous lesions manage to find their way to the appropriate site 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*, but 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 site of injection, resulting in the clinical condition known as CL. Alternatively, the phagocytes can carry them to mucocutaneous junctions, or to the reticuloendothelial tissues, resulting in MCL or VL, respectively. Cutaneous lesions form in most instances, allowing sand flies access to infected host cells at the raised margin.

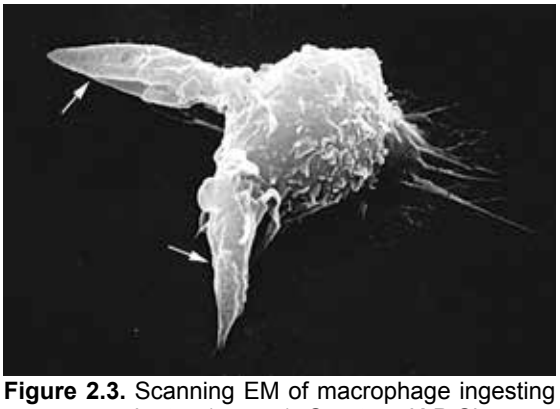

Figure 2.3. Scanning EM of macrophage ingesting two promastigotes (arrows). Courtesy K-P Chang.

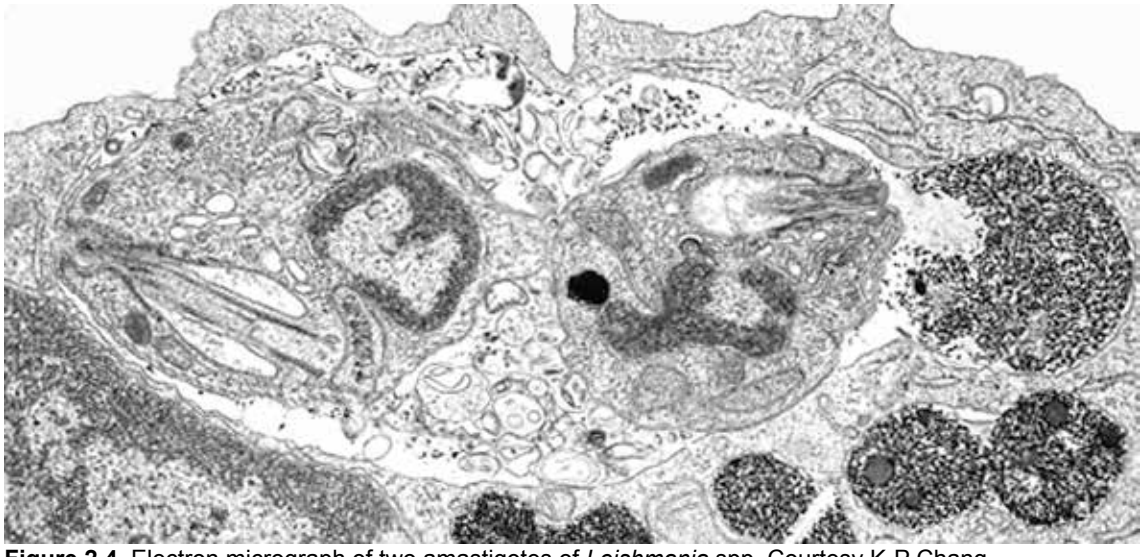

Figure 2.4. Electron micrograph of two amastigotes of *Leishmania* spp. Courtesy K-P Chang.

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 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.[7, 36, 37] 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*.[38]

The following summary of pathogenic mechanisms is derived from both types of experimental approaches. The turning on of heat shock genes, as well as cassettes of other developmentally regulated genes, occurs as the parasite makes the transition from an environment dependent upon ambient temperature (sand fly) to the homeothermic essential niche inside the mammalian host cell.[39, 40] The amastigote down regulates interleukin-12, which delays the onset of cell-mediated protective immune responses.[41, 42] Amastigotes have been shown to interfere with antigen presentation by macrophages, employing cysteine protease B.[43] The amastigote stage also possesses potent cysteine protease inhibitors, which it presumably uses to modify host cysteine protease activity during intracellular infection.[44]

Replication of amastigotes appears to be dependent upon host cyclophilins, since division is inhibited by cyclosporine A.[45, 46] The membrane of the promastigote contains a zinc protease, leishmanolysin, a 63 kDa glycoprotein whose crystalline structure has been determined.[47, 48] Current evidence favors a role for leishmanolysin in migration of parasites through the extracellular matrix after their release from infected cells, by digestion of collagen type IV.[49] Induction of the chemokine MIP-1β by neutrophils harboring amastigotes attracts macrophages to the site of infection. Macrophages then engulf infected neutrophils, thus acquiring the infection.[50]

An exciting advance in our understanding of the severity of certain forms of mucocuta-

neous disease was the discovery of an RNA virus that infects the *L. Viannia* subgenus.[51] It appears that this *Leishmania RNA virus-1* is recognized by host toll-like receptors and induces an inflammatory response that leads to a hyper-inflammatory immune response with resultant destructive lesions.[51]

Protective immune mechanism(s)

The mechanism(s) of protective immunity vary with clinical types of *Leishmania*.[52] 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.[53] Permanent 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.[54] In addition, Langerhans cells are thought to play a major role in antigen presentation and in the induction of interleukin-12 and interleukin-27.[55-57] The main effector mechanism involves cytotoxic T helper cell-dependent macrophage activation and subsequent killing of amastigotes by NO.[58] Chemokines are also important for immunity, and include macrophage inflammatory protein 1β-3β and interferon-γ.[59, 60] Antibodies appear to play no role in immunity to CL, and probably aid the parasite in gaining entrance into the macrophage.[61] 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.[53]

Further complicating the clinical spectrum of diseases caused by *Leishmania*, is how long the *Leishmania* spp. have been around. They have, within the last 165 million years, 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. Thus, when considering the type of disease and the immune responses to them, there exist many exceptions to the above summaries. For an excellent review on this aspect of the biology of *Leishmania* published in 2005, see McMahon-Pratt and Alexander.[62]

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3. Cutaneous Leishmaniasis

Pronunciation: \lēsh-ˈma-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 to understand due to the large numbers of species and clinical variants. Roughly speaking, we consider CL divided along the lines of 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 or not it is zoonotic. Zoonotic species require a significant animal reservoir such as dogs or rodents. Anthroponotic species cycle only or predominantly between humans and sand flies (e.g., *Leishmania (L.) tropica*, (\LEESH-ma-NEE-ah\)).

Old World CL is caused by four species: *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 chagasi, 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 in 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.[1, 2]

Although accurate statistics regarding incidence rates and prevalence are not available, it is estimated that there are 0.7–1.2 million cases of CL each year.[3] Rodents are the primary reservoir for human infection caused by *L. (L.) major*, while domestic dogs serve as reservoirs in many parts of the world for other species of *Leishmania*.[4] Within the last five years there has been an explosion of Old World CL cases linked to the breakdown in public health infrastructure resulting from the conflicts in the Islamic State of Iraq and the Levant-occupied areas of Syria, Iraq, and Libya.[5] Estimates are difficult to come by due

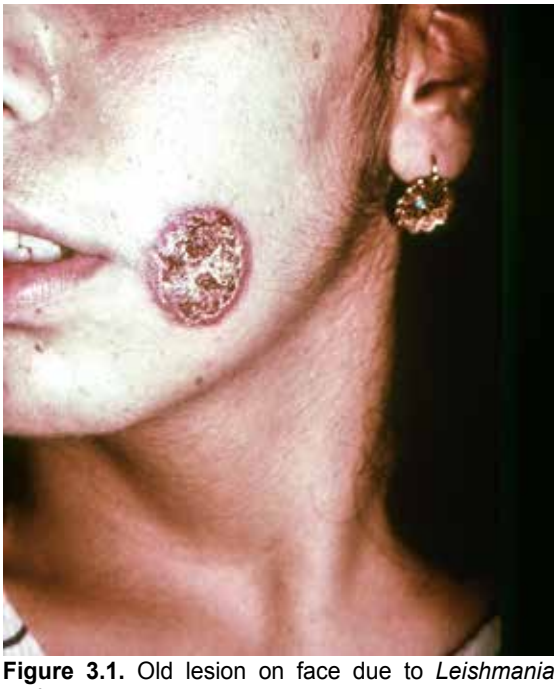
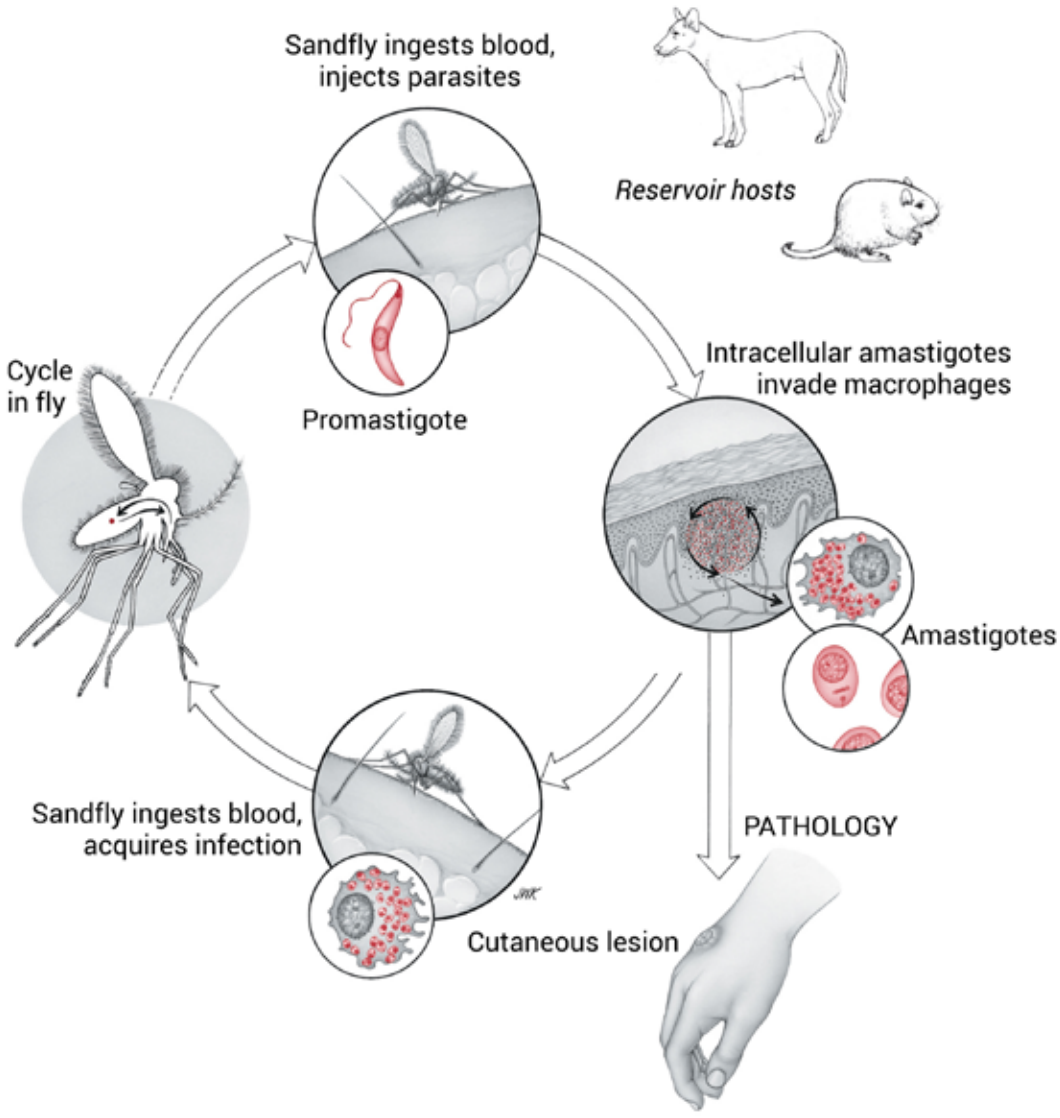


Figure 3.1. Old lesion on face due to *Leishmania major*.

Leishmania tropica



to the absence of public health surveillance in these regions, but it is believed that the numbers could be in the hundreds of thousands. 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.[6, 7]

Historical Information

In 1756, Alexander Russell described the clinical aspects of CL.[8] In 1885, David Cunningham, while working in India, accurately described the *Leishmania* organism he saw in a fixed histological section of a skin lesion.[9] 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 sand flies were the vectors responsible for transmitting *Leishmania* to humans. One species bears their name, *P. sergenti*. Cosme Bueno, much earlier (1764) suspected the same was true for "uta," a cutaneous lesion, later shown to be caused by infection with *Leishmania*.[10]

"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.[11] 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.[12] Unfortunately a significant number of individuals would develop chronic lesions that did not heal as a result of these inoculations, and much effort is being put into alternative approaches.[13]

Life Cycle

Infection begins with the bite of an infected sand fly. The promastigotes are introduced into the subcutaneous tissue, attach to the extracellular matrix and are then taken up by dendritic cells and macrophages. The promastigotes transform into the amastigote stage and begin to replicate. Infection progresses at the site of the bite only. Eventually, a large, painless craterform ulcer develops as the result of extensive cell death.[14] Sand flies acquire the infection by feeding on blood taken up at the margin of the ulcer. [15]

Clinical Disease

CL is first recognized as a small red papule at the site of the bite wound approximately 2–8 weeks after injection of metacyclic promastigotes. The lesion progresses from a painless nodule, measuring approximately 1 cm in diameter, into a much larger one by the formation of satellite papules (Fig. 3.1). The area around the bite wound eventually ulcerates due to intense destruction of cells, and becomes depressed, then heals through scarring (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).

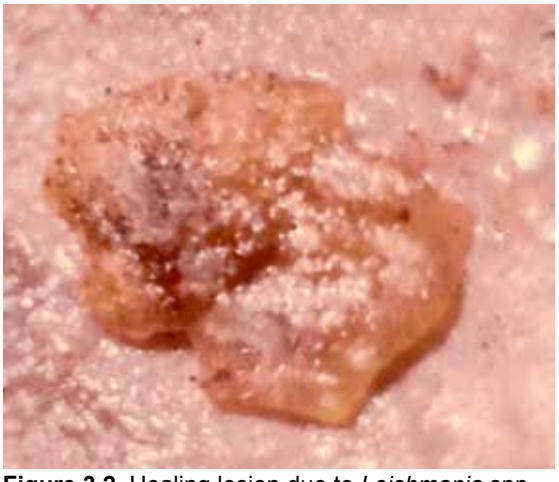


Figure 3.2. Healing lesion due to *Leishmania* spp.

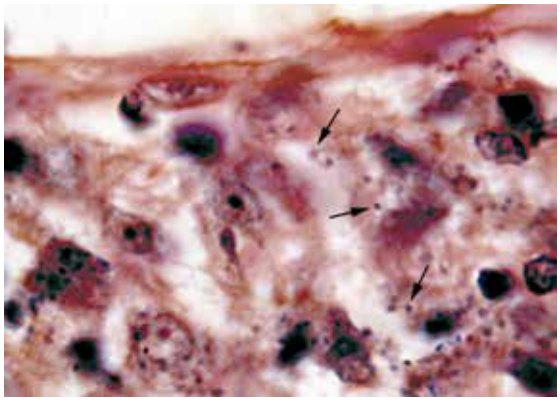


Figure 3.3. Histologic section of skin showing amastigotes (arows) of *Leishmania* spp. in dendritic cells and macrophages.

After the ulcer heals, which may take weeks to months, immunity to reinfection is usually permanent and, although generally species-specific, it may offer some protection against other *Leishmania* species.[12] 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.[16, 17]

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 a lesion that fails to heal is encountered.[18] This is particularly true for CL in the Western Hemisphere.[19] Prolonged infection is the rule in a subset of patients that have an altered pattern of immunity.[20] Chiclero's ulcer, on the pinna of the ear (Fig. 3.5), is a good example of this exception.[21]

Infection due to *L. (L.) aethiopica* is restricted to Ethiopia and Kenya, and causes a wide spectrum of disease, including diffuse cutaneous leishmaniasis (DCL), characterized by involvement of the entire surface of the skin.[22-24] Other species of *Leishmania* (e.g., *L. (V.) braziliensis, L. (L.) amazonensis*) have also been diagnosed in patients suffering from DCL.[25] 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 suffering from various forms of leprosy.[26]

Similarly, *L. (L.) mexicana*, *L (L.) amazonensis and L. (L.) venezuelensis* occasionally cause anergic disseminated cutaneous leishmaniasis (ADCL), which is similar to DCL.[27] 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.[28-30] *Leishmania (L.) infantum* can cause cutaneous lesions but

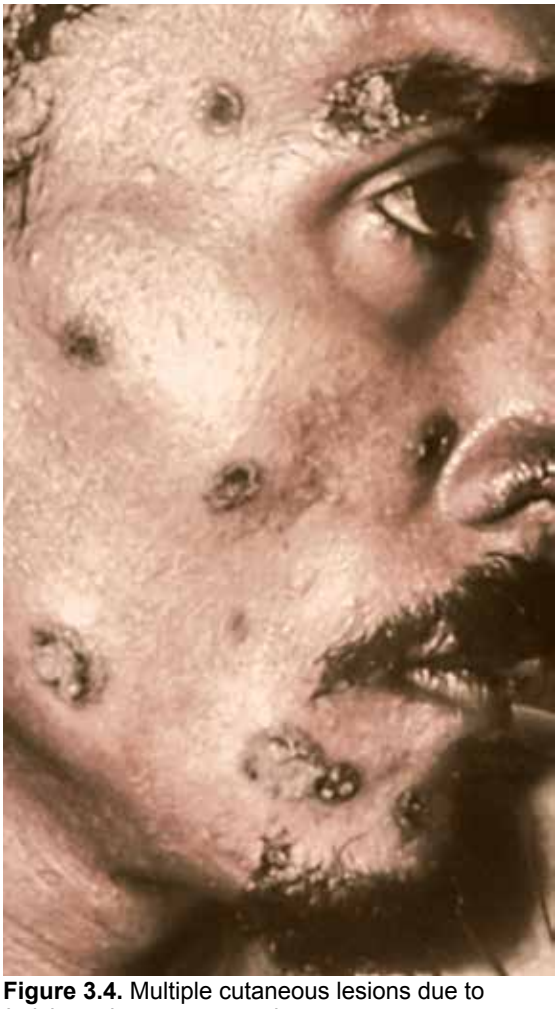


Figure 3.4. Multiple cutaneous lesions due to *Leishmania panamanensis*.

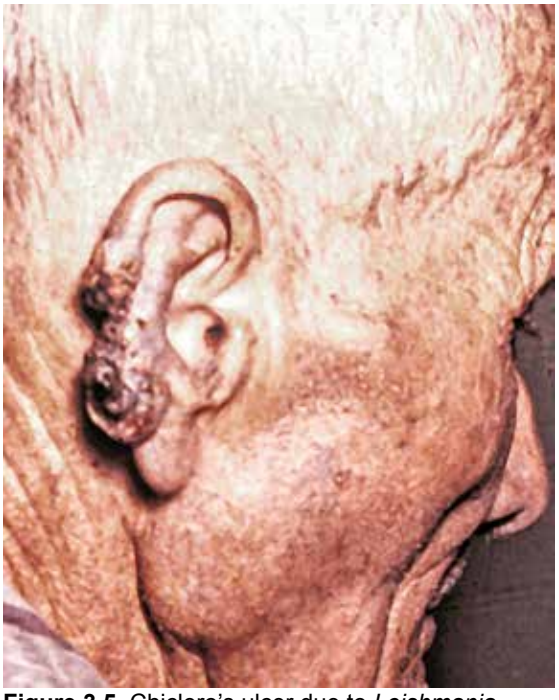


Figure 3.5. Chiclero's ulcer due to *Leishmania*.

is more commonly associated with visceral disease in children throughout the Mediterranean basin. [14]

Patients with HIV/AIDS may present with cutaneous lesions only, or the infection can visceralize.[19] While cutaneous lesions are not frequently described in the HIV-negative population with visceral leishmaniasis (VL), multiple patients co-infected with *Leishmania* and HIV, with cutaneous lesions containing *Leishmania* organisms have been reported.[31]

Diagnosis (see Clinical Appendix)

The typical lesion seen 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., craterform with a raised edge), there are many other dermatological conditions that might be mistaken for CL. Diagnosis of all forms of CL due to *Leishmania* is best achieved with isolation of the organism or through NAAT. Samples should be taken from the margin of an active ulcer that is not obviously superinfected.[32] 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 prior to sending specimens off for histology, culture, microscopy, or NAAT.[32]

NAAT techniques have high sensitivities and also offer the advantage of species identification.[33-37] Species identification is critical in determining whether the infecting species has the potential to progress to mucocutaneous disease.[38] Histopathological examination of biopsy tissue has less sensitivity than NAAT techniques and requires recognition of the characteristic amastigote tissue form of the parasite with the nucleus and kinetoplast (the *dot and dash*).[39] Culture of needle aspiration samples, scrapings and biopsy specimens is a viable alternative to NAAT, but 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 rates of success.[40-42]

Serology is not routinely used in the diagnosis of CL, but in some instances, antibody testing has been used in the evaluation of patients from non-endemic areas.[43] The leishmaniasis skin test is used in certain parts of the world and involves injecting killed promastigotes intradermally and is read at 48–72 hours, much like the purified protein derivative (PPD) skin test for tuberculosis infection.[44] An interferon-γ release assay is also being developed for leishmaniasis.[45]

Treatment (see Clinical Appendix)

Treatment of CL is dictated by the severity of the lesion, its location, and the species involved. Uncomplicated infections that do not involve species that are 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 therapy.[46, 47] In the majority of cases spontaneous healing will occur with proper hygiene and wound care.[48, 49]

Cryotherapy using liquid nitrogen has been successfully employed.[50-52] Treatment with thermotherapy delivered by radiofrequency, superficial heat at 50 °C, or application of heated prongs can result in clinical cure but must be used with caution to avoid nerve damage and burns.[53, 54] 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.[55-57]

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.[58] Systemic therapy is recommended if there is concern that the infecting species has the ability to cause mucosal disease, based on species identification or acquisition of the infection in what is referred to as the mucosal belt (Brazil, Bolivia, and portions of Peru).[59] 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, but increasing rates of resistance has already limited its use.[60] Amphotericin B and its liposomal form are alternative and highly effective options for treatment.[61] In the developed world liposomal amphotericin B is a frequent choice of therapy but its cost is prohibitive in many areas of the world where this disease is endemic. Miltefosine is an effective but expensive option that allows for systemic therapy, its oral dosing is currently FDA approved in the United States for treatment of leishmaniasis.[29, 62-65] Pentamidine is a little-used therapy owing to concerning side effects and limited data on its efficacy.[66] Azoles such as, ketoconazole, fluconazole, itraconazole, and posaconazole are alternative therapies, but ones that show variable efficacy compared to other options based on the particular species of *Leishmania* parasite involved.[55, 67-73]

Prevention and Control

Since humans are a dead-end host in most cases, treatment of infected humans is not expected to have a significant impact on disease transmission.[74] Although dogs have been identified as a significant reservoir, infection in multiple mammals, both wild and domesticated, is not observed.[75] 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.[55] Sand flies only bite at certain times of the day in what is termed a crepuscular/nocturnal pattern: in the morning, late in the evening, and at night.[76] Although avoidance of outdoor activities during these times reduces the chance of being bitten, this can be difficult advice to follow 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 perhaps more viable, approaches to avoid sand fly bites in high-risk areas are the use of insect repellents and insecticide-treated clothing that covers most of the body.[77, 78]

Vaccines, employing combinations of parasite antigens, offer hope that a standard vaccine for use against both CL and VL may eventually be available, but no human vaccine is currently available.[17, 79] A recent health economic study confirmed the cost-effectiveness of a CL vaccine.[80]

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4. Mucocutaneous Leishmaniasis

Pronunciation: \lēsh-ˈma-nē-ə\

Leishmania Viannia braziliensis
(Vianna 1911)

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] Mucocutaneous disease has been observed to develop in 2–10% of patients initially infected with these subspecies, but the risk appears to depend on a patient's gender, age, and nutritional status.[3] Usually following primary infection, but at times coincident with cutaneous disease, the parasites metastasize to mucocutaneous junctions (oral cavity, urogenital, and anal areas), where they erode the soft tissues.[4] This is often a disfiguring condition as it can affect the face and lead to extensive destruction.[5, 6] Since MCL is, in general, restricted to the New World, sand flies of the genus *Lutzomyia* are considered to be the vector.[7] MCL is mainly concentrated in Ecuador, Bolivia, and Northern Brazil, the so-called mucocutaneous belt.[8] A number of the clinical cases of MCL involve *Leishmania* parasites that are infected with a double-stranded RNA virus (Leishmaniavirus), which appears to correlate with disease severity.[9-11]

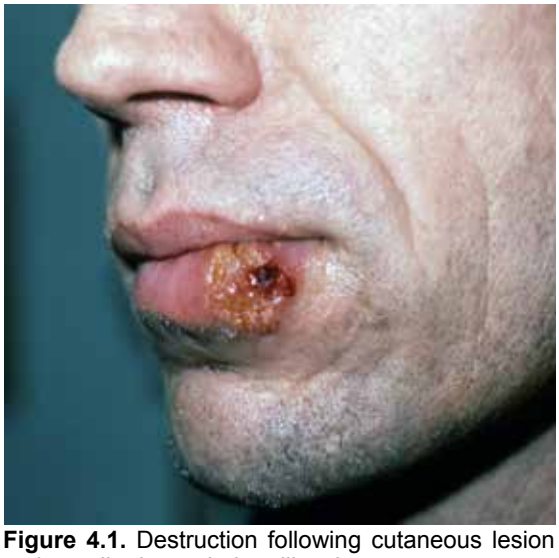


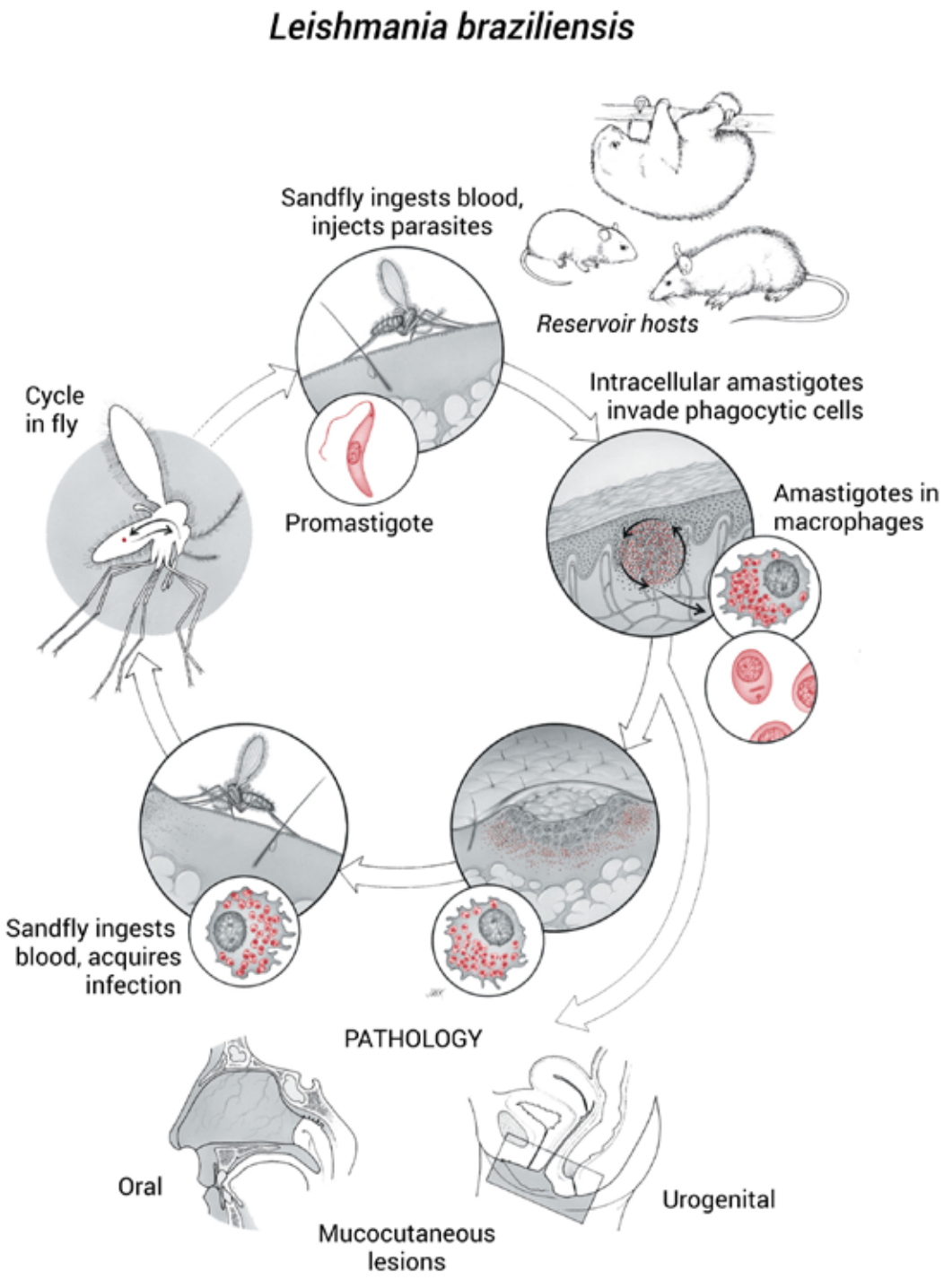
Figure 4.1. Destruction following cutaneous lesion on lower lip due to *L. braziliensis*.

Historical Information

In 1911, Antonio Carini identified patients suffering from mucocutaneous lesions as being different from those only demonstrating cutaneous lesions, thereby establishing MCL as a separate clinical entity.[12] In 1912, the following year, Gaspar Vianna identified and named *L. (V.) braziliensis* as the causative agent.[13] In 1916, Israel Kligler and, in 1926, Hadeyo Noguchi using serological and culture methods, succeeded in characterizing this parasite as a distinct species, despite the lack of modern laboratory equipment.[14, 15]

Life Cycle

The disease is initiated when an infected sand fly takes a blood meal, as with cutaneous 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 goes on to develop MCL.[4] At these distant mucocutaneous sites, the amastigotes divide within resident macrophages and tissue erosion begins to develop. It is likely that this destruction is attributable more to an exuberant host response driven by an exaggerated T-cell response than any fac-


Leishmania braziliensis
Sandfly ingests blood, injects parasites
Reservoir hosts
Cycle in fly
Promastigote
Intracellular amastigotes invade phagocytic cells
Amastigotes in macrophages
Sandfly ingests blood, acquires infection
PATHOLOGY
Oral
Mucocutaneous lesions
Urogenital

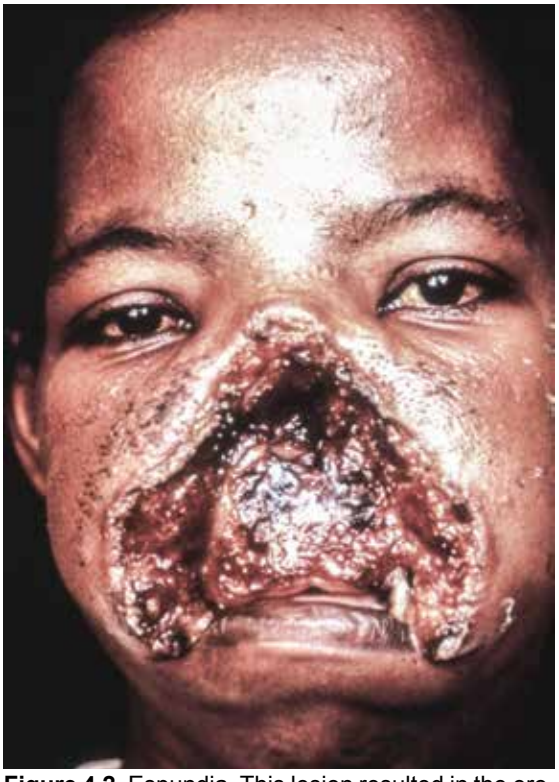


Figure 4.2. Espundia. This lesion resulted in the erosion of the soft palette. Most lesions do not advance this far before medical intervention.

tors intrinsic to the parasite.[2] Perhaps the presence of an RNA virus infecting the responsible *Leishmania* parasites plays a role in augmenting this immune response and driving the hypersensitivity response.[11] 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) which serve as the source of infection.[16]

Clinical Disease

MCL lesions developing in the nasal passage (the most common mucocutaneous site) are characterized by a necrotizing inflammation of mixed cell types (plasma cells and lymphocytes).[5] Skin, mucous membranes, and cartilage can also be involved.[17] Lesions of the oral cavity eventually result in the destruction of the soft palate and nasal septum, as well as invasion of the larynx.[18] When this occurs, the patient may die from infection that spreads to the lungs.[4] MCL is a pauciparasitic disease, and parasites are seldom demonstrated in lesions, although they can be revealed by PCR.[19] 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).[20] Unlike CL, MCL does not usually heal spontaneously.[8, 17]

Multiple cutaneous lesions, as well as extensive mucocutaneous involvement, have been described in patients co-infected with HIV-1.[21-23] Due to enormous genetic variation among strains of *Leishmania* spp. causing MCL, the patterns of disease in rarely occurring MCL infections in HIV-1 patients presents as an exceedingly complex and difficult clinical picture.[24, 25]

Diagnosis (see Clinical Appendix)

Definitive diagnosis is by PCR.[26] Organisms are difficult to find in biopsy of infected palate and soft tissues of the oral cavity, so microscopy of histological sections is not an option for diagnosis. Culture of biopsy material is a possibility (see diagnosis for CL), but growth of promastigotes at room temperature is slow.

Treatment (see Clinical Appendix)

As a general rule systemic therapies are used for the treatment of MCL including pentavalent antimony, amphotericin B, miltefosine, pentamidine, or azole drugs.[8, 27] 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.[28] 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 as well as visceral leishmaniasis (VL). In the developing world, standard amphotericin is often employed for the treatment of MCL if antimonial therapy fails. Miltefosine is an effective but expensive option that allows for systemic therapy with oral dosing that is currently FDA approved in the United States for treatment of leishmaniasis.[29-33] Pentamidine is another option that has demonstrated efficacy in the treatment of MCL.[34, 35] 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.[36-43]

None of the above therapies is 100% effective at eliminating 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.[44]

Prevention and Control

Transmission of *L. (V.) braziliensis* and related species capable of causing MCL occurs through close contact of human populations with reservoir hosts (wild and domestic mammals). Deforestation of vast regions of rainforest for agriculture, mining, and oil exploration, all of which leads to increased urbanization in South and Central America, has exposed large numbers of people to rainforest edges. Edges of natural systems are special ecologically defined areas termed ecotones.[34, 45] 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. Distribution of sand fly species is determined by the availability of natural habitat and of that created by human encroachment. Some species favor unaltered forest, while others thrive in ecologically disturbed situations.[34] It is unfortunately beyond the scope of this text to illustrate this concept with specific examples, but many could be given that would serve to reinforce the idea that when ecological change due to human settlement occurs, those living there are at greater risk from acquiring zoonotic infectious diseases (e.g., Yellow fever virus, Ebola virus, Marburg virus, Lassa fever virus).[45] The number of species of *Leishmania* known to infect humans throughout South America alone has increased dramatically. [4]

Avoidance of contact with vectors of MCL is an ineffective recommendation, given the lack of awareness of their presence on the part of those living in close proximity to them. For those traveling to endemic areas wishing to avoid the bites of sand flies, the following advice may be helpful. Sand flies are more prevalent during the rainy season.[46] Sand flies only bite at certain times of the day in what is termed a crepuscular/nocturnal pattern: in the morning, late in the evening, and at night.[7] 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 perhaps more viable approaches to avoid sand fly bites that are in use by travelers and workers in high-risk areas are the use of insect repellents and insecticide-treated clothing that covers most of the body.[47, 48] Growing rates of insecticide resistance in sand fly populations are already limiting the utility of this approach in many parts of the world.

The use of insecticide-impregnated clothing and bed netting has helped reduce disease transmission for malaria, particularly in Africa, and 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 originally made 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 currently available, despite continued efforts.[49, 50]

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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-ˈma-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 to be the same species as *L. (L.) infantum*.[2, 3] In Europe, Africa, and Asia, both species, *L. donovani* and *L. infantum,* are found in fairly well-defined geographical distributions, while 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] It is currently estimated that each year there are 400,000 new cases and 40,000 deaths due to VL.[4, 12] The majority of disease occurs in a concentrated area in Northern India, Nepal, and Bangladesh.[13]

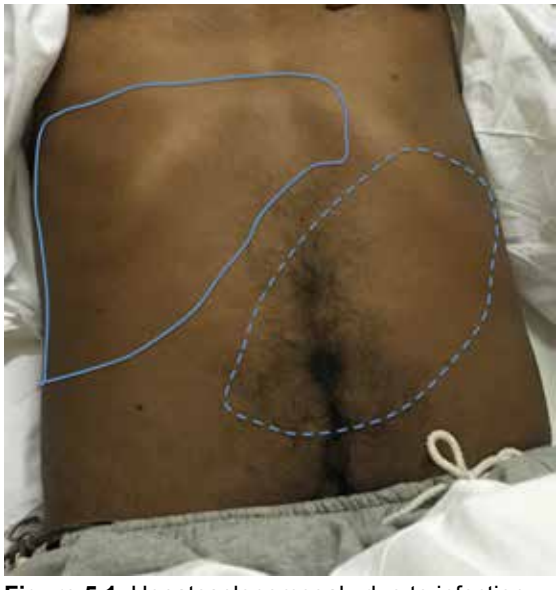
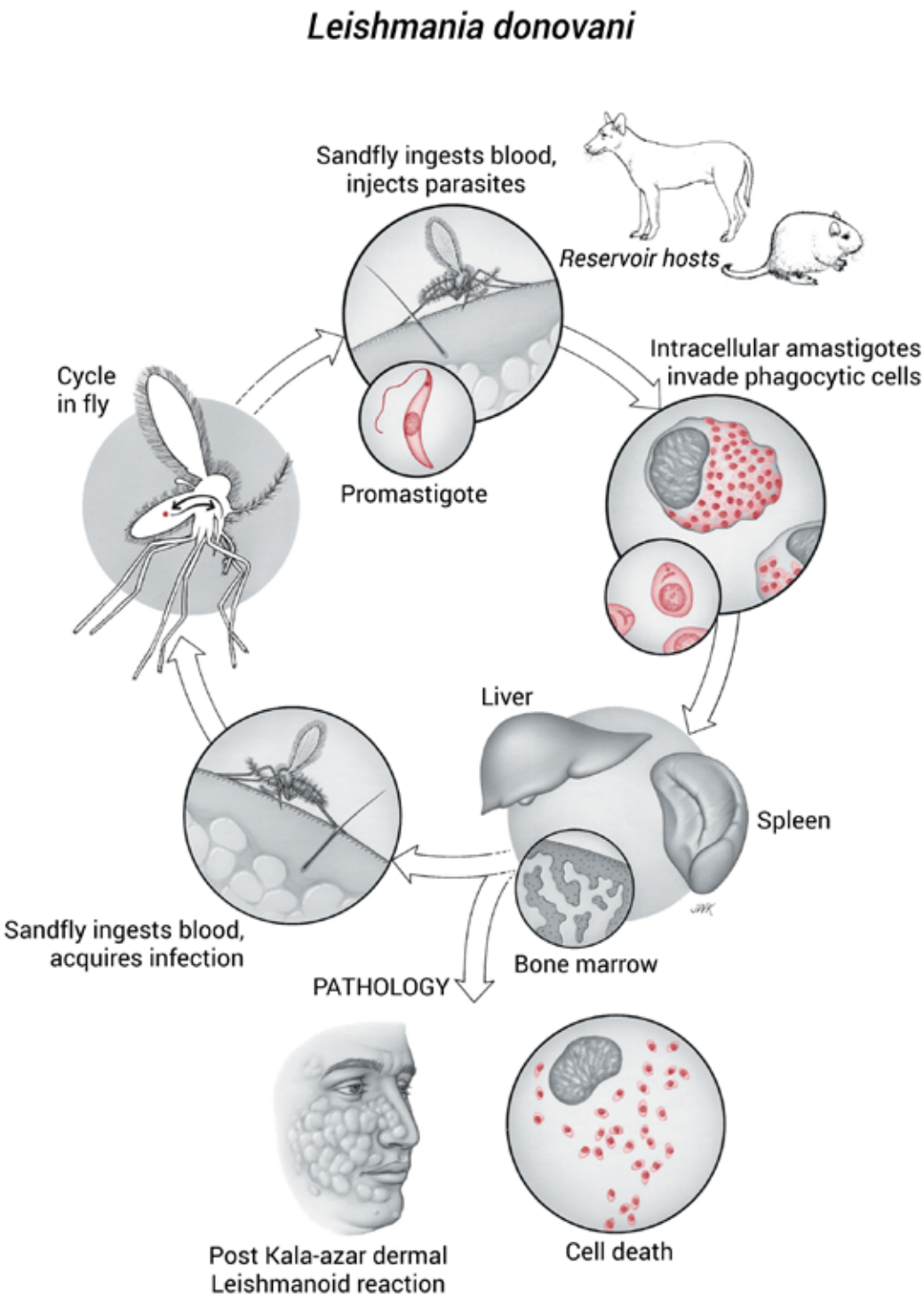


Figure 5.1. Hepatosplenomegaly due to infection with *Leishmania (L.) donovani*.

As with all other species of *Leishmania*, the vectors of VL are numerous species of sand flies. Humans are the primary source of *L. (L.) donovani* infection, although domestic dogs may act as reservoir hosts. In Northern India, Nepal, and Bangladesh the cycle is almost entirely anthroponotic, while 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.

With regard to the presence of VL in the New World, one hypothesis favors *L. (L.) infantum* as having been introduced into the New World several million years ago, since it is found in a fox species native to the remote inner Amazon River basin and causes no disease in that host.[14] This latter point is taken as evidence for its adaptation to that fox host species with reduced virulence. Another theory favors its introduction by the Spanish some 500 years ago during the time of their colonization.[15, 16] In either case, it is found in peridomestic habitats, infecting domestic dogs and humans. In addition, it occurs in a broad range of natural environments, where it infects 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 Hemisphere species of *Leishmania*, despite the fact


Leishmania donovani
Sandfly ingests blood, injects parasites
Reservoir hosts
Promastigote
Intracellular amastigotes invade phagocytic cells
Cycle in fly
Liver
Spleen
Bone marrow
Sandfly ingests blood, acquires infection
PATHOLOGY
Post Kala-azar dermal Leishmanoid reaction
Cell death

that its minicircle DNA shows strong homology with New World species.[17]

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.[18, 19] 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. In 1908, Charles Nicolle discovered that other mammals, particularly the dog, could also become infected.[20] In 1942, C.S. Swaminath and colleagues, working in India, using human volunteers, showed that the infection was transmitted by sand flies of the genus *Phlebotomus*.[21]

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*.[22]

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).[23] The parasites transform into amastigotes and divide, eventually killing the host cell. The released amastigotes circulate or are carried by infected cells to new areas of the body, where macrophages again become infected. The ability of some *Leishmania* spp. to visceralize, in contrast to those species restricted to the skin, may relate to the VL type's survival at higher body temperatures.[24] 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 site of the bite.[23]

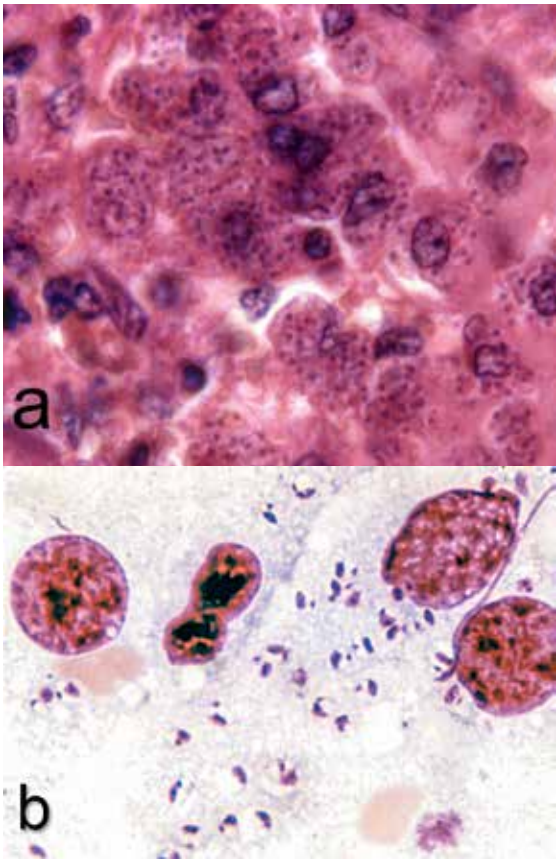


Figure 5.2a. Biopsy of liver positive for amastigotes of *L. (L.) donovani.*
Figure 5.2b. Bone marrow aspirate positive for amastigotes of *L. (L.) donovani.*

The entire reticuloendothelial system eventually becomes involved, compromising the ability of the host 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.[25] Since sand flies bite more frequently at night, this diurnal rhythm makes the acquisition of 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.[25] The duration of the cycle within the fly is approximately 10 days. Rarely, *L. (L.) donovani* has been transmitted by organ transplantation.[26]

Immunity is dependent upon Th1 responses and loss of or decreased Th1 response to *Leishmania* spp. may be critical to development of VL.[27] T cells producing interferon-γ, TNF-α, and interleukin-2 activated macrophages kill intracellular parasites by NO synthesis.[28] 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.[29]

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.[30] Kala-azar is roughly translated as "black fever", because of the appearance of the febrile patient's skin at the height of the infection. Most cases of Kala-azar have an incubation period of 3–6 months.[30] A large number of patients who are infected remain asymptomatic with variable frequency that may be due to parasite virulence, host genetics, nutritional status, and age of infected host.[31-34] When disease does manifest, the onset can be gradual or sudden.[30] 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.[35]

Onset of 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. The infected individual, though weak, does not necessarily feel ill, and tolerates bouts of fever without complaint, distinguishing them from individuals 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 diseases common in the areas where VL is endemic.[36] Generalized lymphadenopathy, and later, hepatosplenomegaly eventually develop. In different areas where VL is seen, the classic darkening of the skin associated with this disease is seen at variable frequencies.[37]

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 is notable in accelerating the overall immune dysfunction when co-infection is present.[38]

As VL progresses patients may develop anemia, low platelet levels (thrombocytopenia), hepatosplenomegaly, and a drop in hemoglobin.[36] High concentrations of serum protein, approaching 10 g/dl, is due almost exclusively to a rise in non-protective IgG resulting from a polyclonal activation of B cells.[29, 39] 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 involvement of the reticuloendothelial system.[40, 41]

Congenital Kala-azar

Infants born to mothers with Kala-azar can acquire the infection *in utero*.[42] Organisms are brought to the developing fetus by infected macrophages. The pathology and clinical presentation of congenital VL in newborns is similar to the adult form.[43]

Post-Kala-azar Dermal Leishmaniasis

A number of 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).[44] 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.[45] Among African patients, only 2% of those who recover develop such skin lesions. Involvement of the eye is also common.[46] PKDL is strongly associated with an interleukin-10-driven (Th2) cytokine pattern.[47-49] Individuals with PKDL are a significant concern in areas where anthroponotic transmission is significant, as these individuals are felt to be highly efficient spreaders of disease.[12]

Atypical CL has also been described for infections caused by *L. (L.) infantum*; it may either be a form of PKDL following a subclinical infection, or a form of Kala-azar that presents with solely cutaneous manifestations.[50]

Diagnosis (see Clinical Appendix)

Definitive diagnosis of VL depends upon isolation of the organism or NAATs.[51-53] PKDL can be diagnosed by NAAT with high sensitivity.[54] 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.[55-58] Culture of bone marrow aspirates is effective at revealing the organisms, but it takes three to four days for the parasites to grow to significant numbers. 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.[59, 60] A simple point of care lateral flow rK39 assay is now utilized in many endemic areas.[61]

Treatment (see Clinical Appendix)

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.[62] The liposomal form of amphotericin is better tolerated, has a better safety profile, improved bioavailability compared to amphotericin, and is taken up by the phagocytic cells that are infected by the *Leishmania* amastigotes.[63]

Sodium stibogluconate is preferred for use in East Africa for Kala-azar patients, but resistance limits its use as an alternative in India and resistance rates must be monitored elsewhere.[64, 65] Continued use of antimonials currently depends on regional resistance rates (high in India), and access to alternative therapies.[62] Miltefosine is an oral agent available as a secondary choice for systemic treatment of VL, and further studies will help define its role in treatment of childhood VL.[66, 67] Miltefosine may be an important part

of short course combination treatment with liposomal amphotericin B, and is being studied.[68] Pentamidine and sitamaquine (another oral agent) are alternatives that may end up having a role in therapy of VL.[62] Response to any therapy is generally assessed clinically with resolution of fever, reduced splenic size, and weight gain.

Prevention and Control

Infection due to *L. (L.) donovani* and *L. (L.) infantum* differ from one another 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 which has been eliminated.[69, 70] Despite these challenges there is a Gates initiative underway attempting to achieve elimination in the Indian subcontinent.

Political unrest, forced migration due to wars (primarily in Africa and Afghanistan), and prolonged periods of drought (primarily in India) have resulted in several situations where large numbers of patients have become afflicted with Kala-azar. These situations concentrate people into 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 have met with a singular lack of success, as is also the case throughout South and Central America. Leishmaniasis due to *L. (L.) infantum* in domestic and wild dogs has been described in the United States.[71] No human cases have been reported from this area of the world yet, but the proper vector species of sand flies are present, and perhaps it is just a matter of time before a human outbreak occurs.

Research to develop a human *Leishmania* vaccine is longstanding yet confounded by host status, multiple species and our lack of understanding of what constitutes immune protection.[72] A canine vaccine exists and given recent advances in novel adjuvant development together with improved antigen preparations, this may provide hope for progress toward a human vaccine.[73] Remote sensing efforts have identified rainfall and altitude as the two most important environmental variables in predicting outbreaks of VL in certain parts of the world.[74] It is hoped that future studies using this powerful new technology will result in an even greater application of satellite data to augment the control of leishmaniasis in other parts of the world as well.

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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-ˌpan-ə-ˈsō-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 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. Both types of HAT are spread by the bite of the tsetse fly. 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 major outbreaks, due mainly to extensive forced migrations caused by civil turmoil leading 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] *T. brucei* and related species are part of a larger group of organisms characterized by the presence of a kinetoplast (a primitive mitochondrion), and 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 in Uganda where both forms are present.[2]

In West Africa, the domestic pig is considered the most important reservoir host for *T. b. gambiense*.[3] In contrast, many species of wild animals and domestic cattle of 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.[4, 5] Trypanosomiasis is also a serious problem in domestic animals imported to Africa from Europe. In addition, many species of the try-

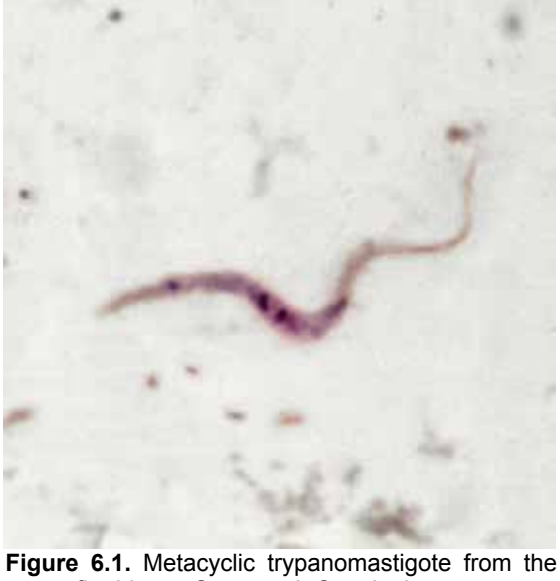
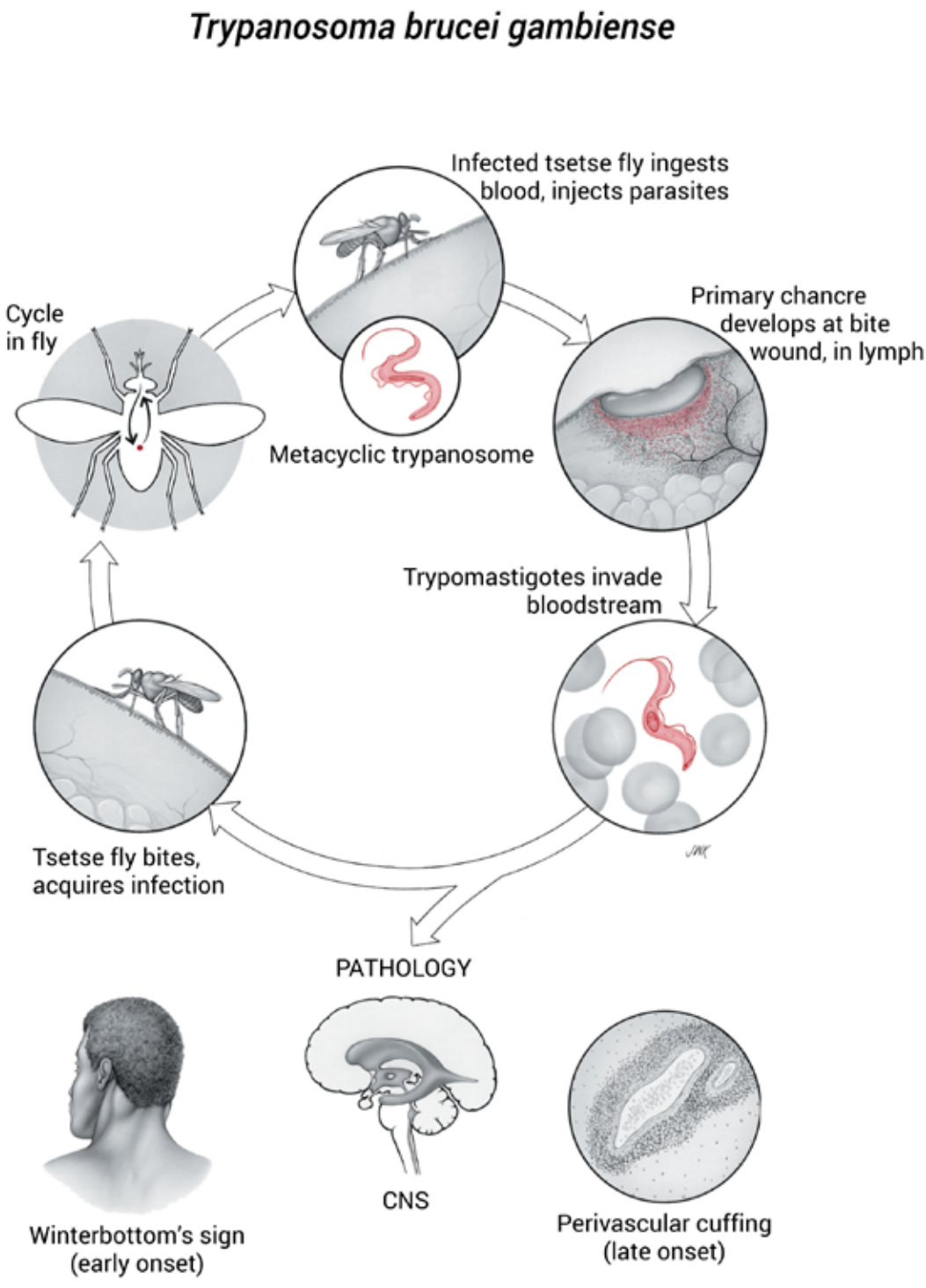
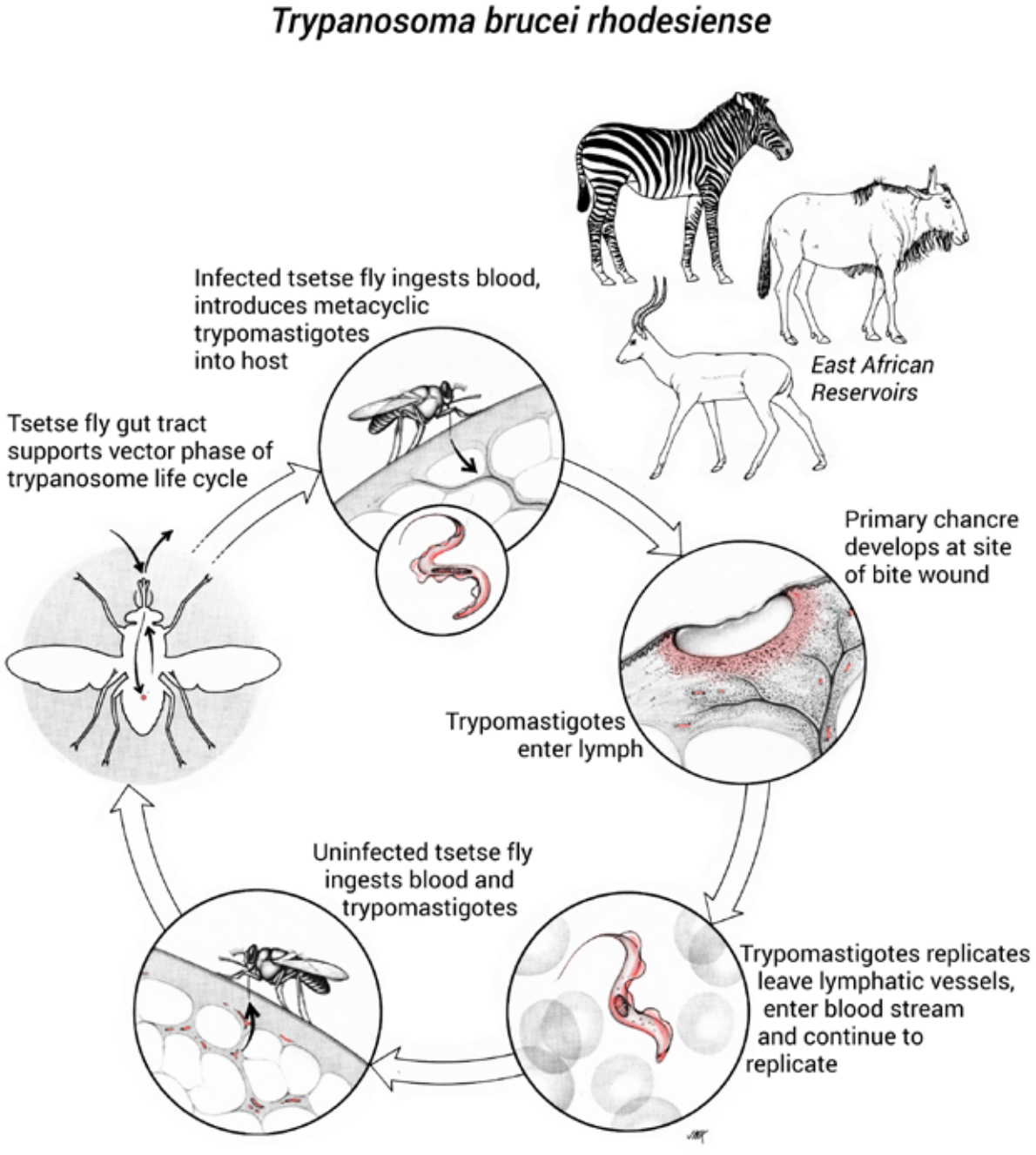


Figure 6.1. Metacyclic trypanomastigote from the tsetse fly. 20 μm. Courtesy I. Cunningham.


Trypanosoma brucei gambiense
Infected tsetse fly ingests blood, injects parasites
Primary chancre develops at bite wound, in lymph
Trypomastigotes invade bloodstream
Tsetse fly bites, acquires infection
Cycle in fly
Metacyclic trypanosome
PATHOLOGY
Winterbottom's sign (early onset)
CNS
Perivascular cuffing (late onset)


Trypanosoma brucei rhodesiense
East African Reservoirs
Infected tsetse fly ingests blood, introduces metacyclic trypomastigotes into host
Tsetse fly gut tract supports vector phase of trypanosome life cycle
Primary chancre develops at site of bite wound
Trypomastigotes enter lymph
Uninfected tsetse fly ingests blood and trypomastigotes
Trypomastigotes replicates leave lymphatic vessels, enter blood stream and continue to replicate

panosomes related to those infecting humans cause severe disease in cattle.[6,7] The genomes of several trypanosomes have now been sequenced.[8-10]

Historical Information

Sleeping sickness was described in the 1700s, when John Atkins published his observations of the disease.[11] 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.[12] In 1902, Robert Forde, working in West Africa, described a clinical condition in humans similar to that in cattle caused by *T. b. gambiense*.[13] In 1910, John Stephens and Harold Fantham isolated and described *T. b. rhodesiense* from human cases in East Africa.[14] The two organisms, *T. b. gambiense* and *T. b. rhodesiense*, are morphologically identical. In 1912, Allan Kinghorn and Warrington Yorke demonstrated that *T. b. rhodesiense* could be transmitted from humans to animals by tsetse flies.[15] 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 4 years in Africa. Upon returning to England to take over his father's medical practice, Dr. Winterbottom published a description of diseases he had seen including West African trypanosomiasis and noted that the slave traders would avoid taking slaves with the characteristic posterior cervical adenopathy that is known today as Winterbottom's sign.[16]

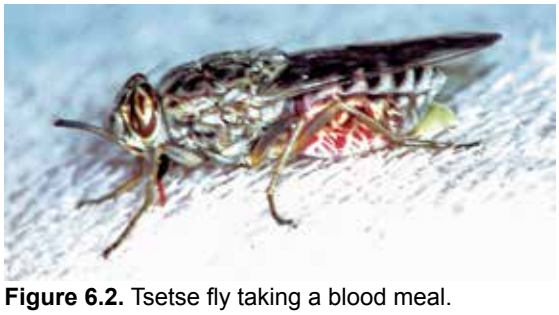


Figure 6.2. Tsetse fly taking a blood meal.

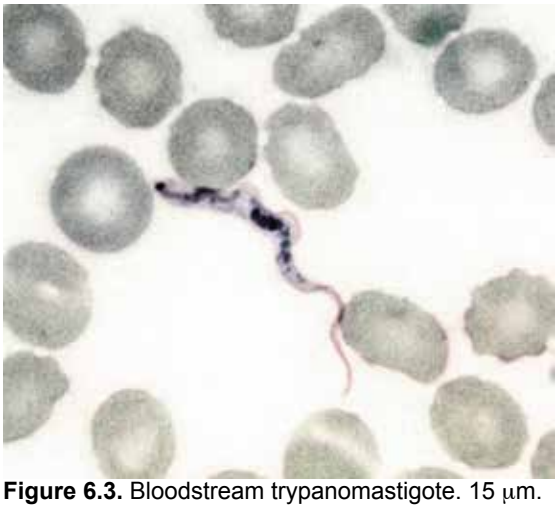


Figure 6.3. Bloodstream trypanomastigote. 15 μm.

Life Cycle

Biological characteristics of the two subspecies are very similar. African trypanosomes live extracellularly, both in the mammalian and insect host.[17] 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, 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 that is dependent on a host T-cell response.[18] 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.[19-23] 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.[24-26] Maxi circle DNA contains sequences that, when edited, direct the translation of mitochondrial proteins.[27, 28]

In the vertebrate host, trypanosomes depend entirely upon glucose for energy and are highly aerobic, despite the fact that 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.[29]

The surface of the trypanosome has numerous membrane-associated transport proteins for obtaining nucleic acid bases, glucose, and other small molecular weight nutrients.[30, 31 32] These essential proteins are shielded by allosteric interference provided by the variant surface glycoproteins (VSGs).[33]

The trypomastigote enters the bloodstream through the lymphatics and divides further, producing a patent parasitemia. The number of parasites in the blood varies with 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. At some point, trypanosomes 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.[34]

Both male and female tsetse flies become infected by ingesting a blood meal from an infected host.[35] 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.[36] The trypanosomes then develop into procyclic trypomastigotes in the midgut of the fly, and continue to divide for approximately 10 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 tsetse, the strain of the trypanosome, and the ambient temperature.[35] If tsetse flies ingest more than one strain of trypanosome, there is the possibility of genetic exchange between the two strains, generating

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

an increase in genetic diversity.[37]

The vector remains infected for life (2–3 months for females). 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.[38] 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 nonhuman 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 molecular strategies enabling them to avoid elimination from the mammalian host. In addition to antigenic variation, certain genotypes of trypanosomes have the ability to survive in the presence of high levels of interferon-γ, while other genotypes have the ability to avoid complement-mediated destruction.[39, 40] In some cases, patients are infected with multiple genotypes that display multiple mechanisms of immune evasion.[41]

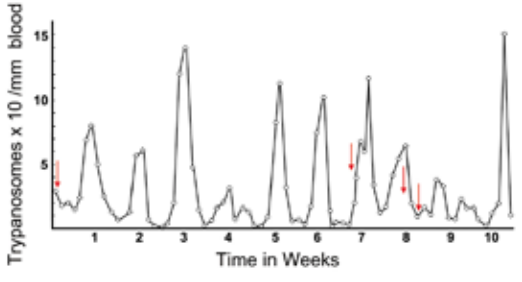


Figure 6.5. Parasitemia in a patient infected with *T. b. rhodesiense*. Each peak of parasitemia represents a new antigenic variant. Arrows indicate attempts at chemotherapy. Ultimately, the patient died of overwhelming infection.

All infected mammals produce antibodies against a membrane-associated antigen of the trypanosome referred to as the variant surface glycoprotein (VSG).[42] Specific IgG antibodies destroy all clonal organisms sharing the same surface protein (e.g., VSG-1) by agglutination and lysis. However, a few of the 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 prior to encountering the antibody, and continue to synthesize VSG-2 exclusively, they escape lysis and replace those that were destroyed.[43, 44] 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).

Antigenic variation depends upon trans-splicing of mRNAs encoded by genes that have been rearranged, duplicated, and expressed at a unique site in the genome of the trypanosome.[45, 46] In experimental animal models, the repertoire of antigenic variants of the bloodstream trypomastigotes is large, with observed VSGs numbering in the hundreds. In human disease, the maximum number of VSGs that can be produced remains unknown, although the genome codes for about 1000. Antigenic variation is the primary reason why vaccine development against this pathogen has not progressed.[47]

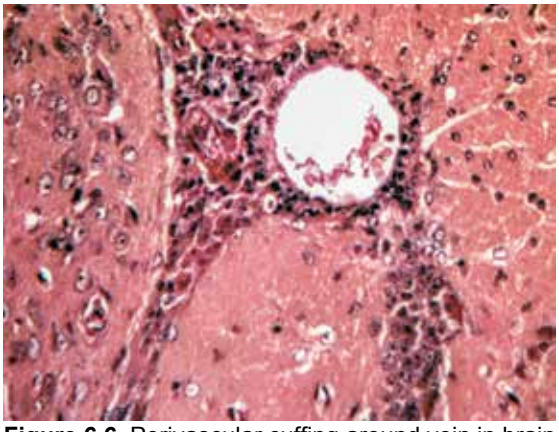


Figure 6.6. Perivascular cuffing around vein in brain of patient who died of sleeping sickness.

Neuropathology

Trypanosomes remain in the bloodstream and lymph nodes throughout the infection period, which can last weeks to years, depending upon the subspecies of parasite and the immune capabilities of the infected individual. All nodes become enlarged, but enlargement of the posterior cervical nodes is the most noticeable.[48] This cervical lymph node enlargement is known as "Winterbottom's sign". The invasion of the CNS induces a lethargic condition, leading eventually to coma and death.[49] 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.[50] 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.[51] Anti-inflammatory interleukins (interleukin-10 and transforming growth factor-β) are produced early on in the infection, but lose their effectiveness during the chronic and late phase.[52]

Clinical Disease

The African sleeping sickness caused by the two species of trypanosomes are different in certain ways. A large, painless chancre (Fig. 6.7) containing the dividing organisms usually develops at the site of the bite within 2–5 days, and subsequently heals.[18] The chancre is more often described in *T. b. gambiense* than in *T. b. rhodesiense,* but 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.[53] Winterbottom's sign is characteristic but not always present.[48]

Infection rapidly progresses on to disease with *T. b. rhodesiense*, with an incubation period of only 2–3 weeks, and a course of 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 indi-

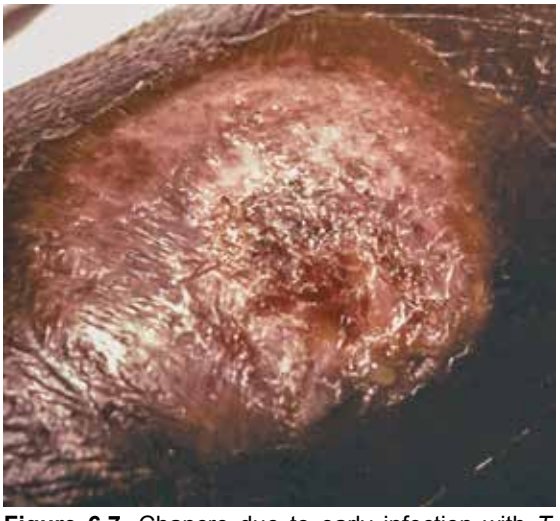


Figure 6.7. Chancre due to early infection with *T. b. gambiense*. Courtesy World Health Organization.

cating some degree of tolerance in African populations.[54] Both forms of sleeping sickness are characterized by two defined stages. The early stage is known as the hemolymphatic stage, and the late stage is known as the CNS stage. During the early stage, there is intermittent fever, malaise, and joint pain that probably correlates with waves of parasitemia. It is during this time that generalized enlargement of the lymph nodes occurs and hepatosplenomegaly may occur.[55] This early stage lasts on average for 3 years with *T. b. gambiense* and for only weeks or months with *T. b. rhodesiense*.[56]

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)

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. Coinfection with malaria may occur given the geographic overlap of the two parasites; however, this should not divert the clinician from the diagnosis of 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.[57] A number of screening programs in endemic areas rely on an initial serological screening test performed using a card agglutination test for trypanosomiasis.[58, 59] Antigen detection testing is currently not routinely employed due to concerns about specificity with currently available tests.[60] Molecular testing is another approach that has been developed, but this approach is still not widely used in endemic areas.[61, 62] 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.[63]

Examination of CSF is mandatory in the diagnostic evaluation of trypanosomiasis and a white blood cell count $\geq$5 cells/μL is considered indicative of CNS involvement.[64, 65] Large plasma cells (Mott cells) containing eosinophilic inclusions are uncommon but characteristic and likely represent cells with large amounts of IgM that they are unable to secrete.[66, 67] The determination of CNS involvement is important, as this will guide the choice of appropriate therapy.

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

Treatment (see Clinical Appendix)

Critical for selection of the appropriate treatment for both East African and West African trypanosomiasis is the determination of the clinical stage of disease: early (hemolymphatic stage), versus late (encephalitic stage), and identification of the species of trypanosome. [68, 69]

For treatment of West African sleeping sickness (*T. b. gambiense)* the current recommended therapy for early stage infection is IV or IM pentamidine.[70] An alternative therapy for early hemolymphatic stage infection with *T. b. gambiense* is suramin, which is equal in efficacy to pentamidine.[70] Suramin has been associated with a hypersensitivity reaction so a test dose should be given before starting treatment with this agent.[71] Suramin as also the first line therapy for early stage East African sleeping sickness (*T. b. rhodesiense*). In early hemolymphatic stage East African sleeping sickness, suramin is the preferred therapeutic agent.[72, 73] Suramin is only effective in the early stages of the infection as it does not cross the blood-brain barrier.[68]

There is significantly more difference in recommendations for therapy of late or encephalitic stage infection for these two protozoan parasites. For West African trypanosomiasis, infection with *T. b. gambiense*, combination therapy with eflornithine and nifurtimox or monotherapy with eflornithine is recommended.[74] For East African trypanosomiasis, infection with *T. b. rhodesiense*, melarsoprol is the treatment of choice. Unfortunately, melarsoprol is the only effective drug for treatment of *T. b. rhodesiense* with CNS involvement, and this toxic drug is associated with encephalopathy in about 3% of cases.[75] Eflornithine, although an effective therapy for late stage with *T. b. gambiense* infection, is not an effective therapy against *T. b. rhodesiense*.[76]

Prevention and Control

Previously, periods of political upheaval in different parts of Africa have resulted in dramatic increases in human cases of sleeping sickness.[77] A 2005 World Health Organization report indicated at least 450,000 new cases that year, alone, while prior to 1995, the estimate was fewer than 70,000.[78] 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 a number of parasitic infections. Tsetse fly control programs are also compromised in these same regions due to political and economic instability, exacerbating an already intractable situation. *T. b. gambiense* may exist in a latent state in humans and under conditions of stress there may be activation, playing a role in some outbreaks.[79] In addition to all this turmoil, HIV/AIDS has complicated the picture, adding new, dimensions to the general problem of disease control. 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 do not obey political boundaries and thrive in certain disturbed environments.[80] Despite these challenges there is an elimination initiative underway led by the World Health Organization.

Work on vaccines based on VSG antigens has not progressed; however, there are a number of active investigations into further understanding the immune response and what constitutes protection from trypanosomiasis.[81-83] Other protein antigens, particularly transporters on the membrane of the flagellar pocket and tubulin, offer promise.[84] Diagnostic tests, other than microscopy, would help in earlier patient diagnosis and control efforts, particularly when there are low parasite numbers and in latent infections.

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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-ˌpan-ə-ˈsō-mə\ \crew see\

Introduction

Trypanosoma cruzi (\try pano so ma\\crew see\) is the causative agent of American trypanosomiasis, also known as Chagas (\shägəs\, \chägəs\) disease. It is an intracellular parasite for the majority of its life cycle, in contrast to 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, where according to the Global Burden of Disease study it infects approximately 6-7 million people.[10-12] A health economic assessment indicates that Chagas disease results in over $7 billion in economic losses annually.[13] According to the World Health Organization, Argentina has the largest number of people living with Chagas disease, followed by Brazil and Mexico, but Bolivia has the world's highest prevalence rate.[14] It is estimated that there are currently more than 300,000 individuals living in the United States infected with Chagas disease.[15] There is strong evidence that transmission occurs within Texas, where dogs are reservoir hosts.[16] Through globalization, Chagas disease cases are also now found in Southern Europe (especially Spain and Portugal), and even Australia and Japan, although there is no disease transmission in these areas.[17] As demonstrated by a study in Brazil, the incidence of this disease varies greatly based on location, from 0% to above 25%.[18]

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.[19] 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%.[20] In individuals who already exhibit signs and symptoms of heart disease, the five-year mortality has been estimated at around 17%.[21]

In addition to spread by the kissing bug (*Triatomine*), infection can be transmitted through blood transfusion, bone marrow transplants, organ transplants, transplacentally, and ingestion of contaminated beverages.[22-27] 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. [28] Contamination is now linked to several outbreaks involving guava juice, sugar cane juice, food, water, soup, and fruit juices. [28-30] Another transmission route of increasing importance is vertical transmission of *T. cruzi* infection from mother to

Figure 7.1. Kissing bug nymph, feeding.

child.[31] Rats, dogs, sloths, bats, and various non-human primates are important reservoir hosts, depending upon the region.[32-37] Transmission takes place in both rural and urban settings. Incidence is highest in children, with the notable exceptions of Brazil and Chile.[38]

Historical Information

In 1909, Carlos Chagas (\shä-gəs\, \chägəs\) observed the infective stage of *T. cruzi* by chance while conducting a survey for vectors of malaria.[39, 40] 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 as well 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. In 1912, Alexandre Brumpt, completed the description of the life cycle of *T. cruzi*, while, in 1916, Gaspar Vianna published the details of the pathological consequences of infection with this important pathogenic protozoan.[40, 41]

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*. Because of this encounter, much speculation has centered on the possibility that Darwin actually contracted and suffered from chronic Chagas disease. In fact, he most likely suffered from lactose intolerance masquerading as Chagas disease![42]

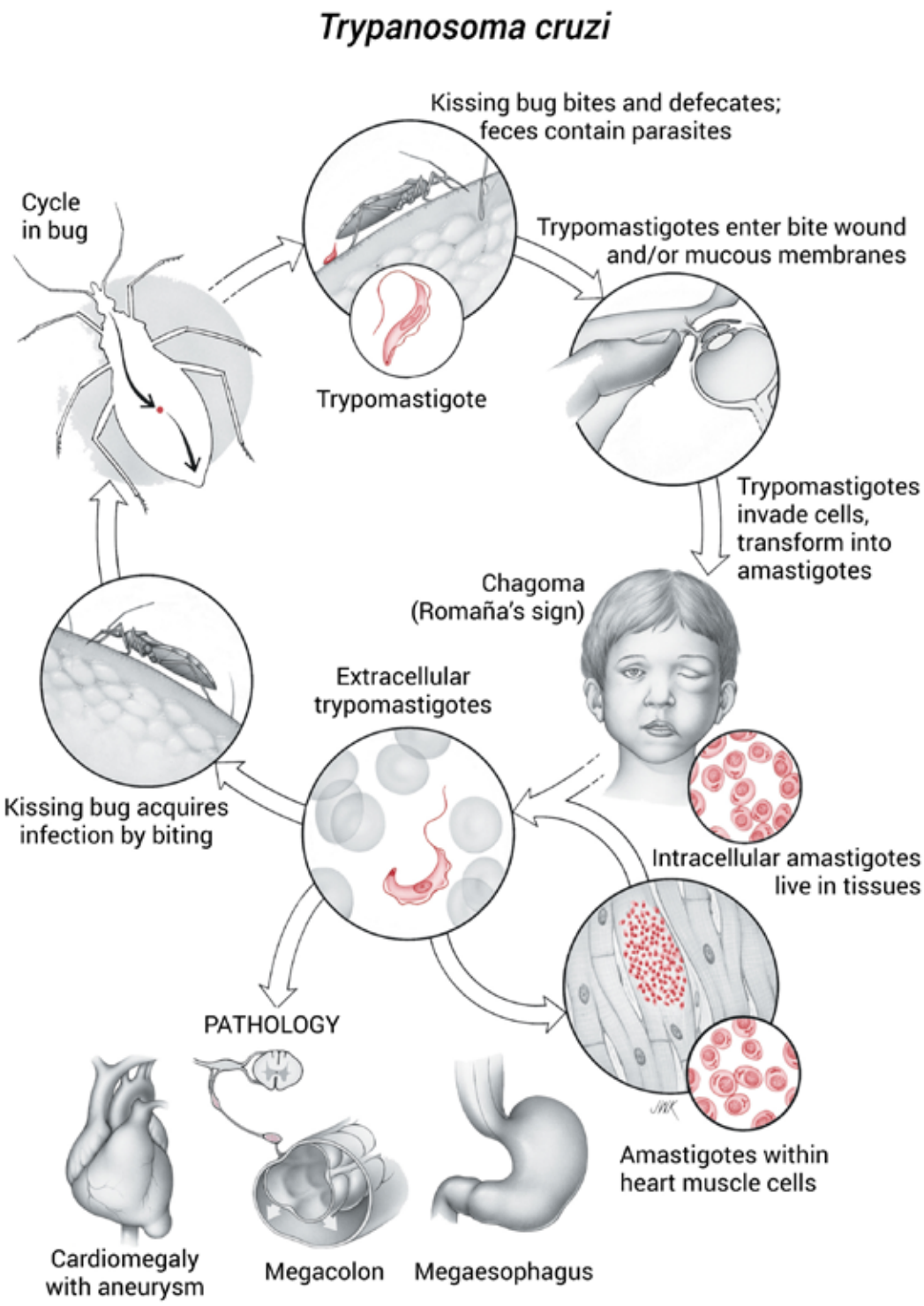
Life Cycle

The biology, molecular biology, and epidemiology of American trypanosomiasis are starting to be revealed at the genetic level.[43-45] 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 simultaneously defecates, depositing feces adjacent to the bite wound. 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 they are asleep. The reduviid bug is often called the "kissing bug" because the bite is painless and it usually bites around the mouth or eyes.[46] The bug's saliva contains antigens that induce intense puritis.[47]

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 the bugs (Fig. 7.2), and their feces have the opportunity to fall onto people while they 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 of infection by this route is high, because

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


Trypanosoma cruzi
Kissing bug bites and defecates; feces contain parasites
Cycle in bug
Trypomastigotes enter bite wound and/or mucous membranes
Trypomastigote
Trypomastigotes invade cells, transform into amastigotes
Chagoma (Romaña's sign)
Extracellular trypomastigotes
Kissing bug acquires infection by biting
Intracellular amastigotes live in tissues
PATHOLOGY
Amastigotes within heart muscle cells
Cardiomegaly with aneurysm
Megacolon
Megaesophagus

kissing bugs feed on many mammals, and rural peoples live in close proximity to 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.[48] The parasite protein that binds to galactin-3 has yet to be identified. The trypomastigote can penetrate a wide variety of cells, and the process is mediated by calcium ions and at least two parasite membrane proteins; a neuraminidase/trans-sialidase, which binds to sialic acid, and penetrin, which binds to heparin sulfate.[49, 50] Another protein, gp82 might also be necessary for penetration into gastric epithelium if the metacyclic trypomastigote stage is swallowed.[51] Just such an outbreak occurred in Santa Catarina, Brazil, involving the ingestion of sugar cane juice contaminated with at least one infected kissing bug. Animals can become infected by ingesting infected kissing bugs, and this might be the usual way for them to acquire the infection.[52]

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.[50] The organism also produces a number of proteins that offer it additional advantages once inside the host cell. Chagasin is a cysteine protease inhibitor and is apparently necessary for avoiding lysosomal-derived cysteine protease activity and ensures that the parasite has the time needed to differentiate into the amastigote stage.[53] Cruzipain is thought to play a major 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.[54] 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, 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.[55] The trypomastigote migrates to the midgut of the insect, where it transforms into the epimastigote, and then undergoes many divisions. Thousands of organisms are produced within one insect without apparently significantly affecting it. The triatomines remain infected for life (~1–2 years). Epimastigotes maintain their place in the gut of the insect by specific receptor-ligand interactions involving at least one parasite surface glycoprotein and a carbohydrate lectin on the gut cells of the insect.[56] Ultimately, 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 of time.[57, 58] For example, *in vitro* culture of human dendritic cells infected with *T. cruzi* resulted in a dramatic down-regulation of synthesis of: interleukins 6 and 12, TNF-α, HLA-DR, and CD40, and inhibited their maturation into antigen processing cells.[59] Parasite-derived calreticulin may also be impor-

tant for amastigote survival in the intracellular environment, implicating a central role for calcium trafficking and storage in the life of the parasite.[60]

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 an answer for this defense strategy. 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.[61]

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.[62] Parasites are killed by way of induction of NO synthase and the production of NO.[63] $CD8^+$ T cells with specificities for parasite antigens are thought to be essential in maintaining some control of the infection throughout the chronic phase.[64]

Chagas disease manifests in many organs. Infected individuals remain infected for life and most of the pathological consequences 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 is directly resultant on damage that occurs early in infection and is not thought to be prevented by treatment of chronic Chagas.[65] Megacolon and megaesophagus are late-onset sequelae to chronic infection. Heart damage is almost invariably associated with Chagas disease in some regions of Central and South America, and is detectable early on in infection.[66, 67] Erosion of heart tissue is typical, and in many cases results in aneurysm and heart failure (Figs. 7.4, 7.5).

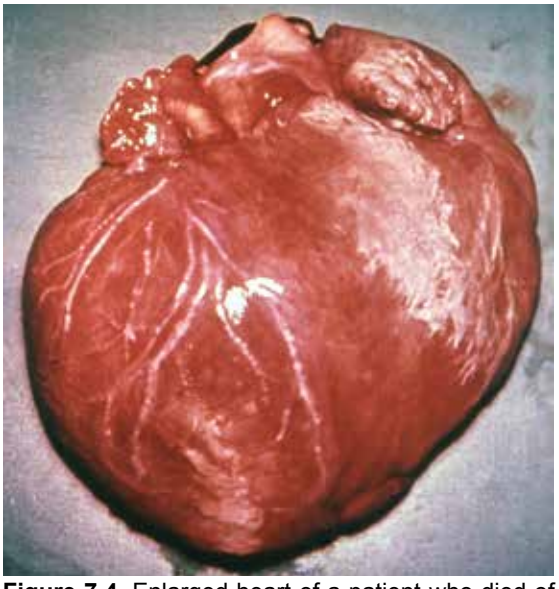


Figure 7.4. Enlarged heart of a patient who died of chronic Chagas disease.

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

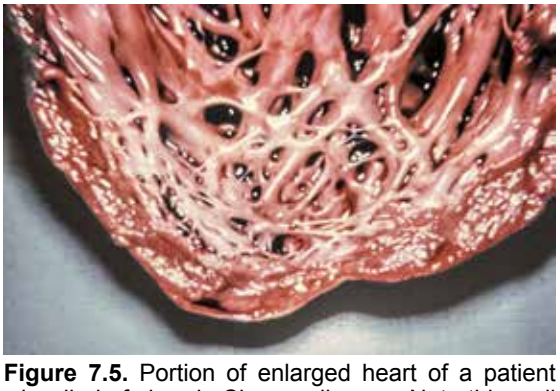


Figure 7.5. Portion of enlarged heart of a patient who died of chronic Chagas disease. Note thin wall of ventricle.

Current thinking regarding a dominant role for auto-antibodies inducing cardiomyopathy plays down this mechanism to account for heart damage during chronic infection.[67] This is because PCR has demonstrated the presence of *T. cruzi* in heart tissue, even at times when biopsy material used in conventional histological mode could not reveal the presence of the parasite. Also, disease progresses rapidly when parasites are abundant and not as fast when they are hard to demonstrate on biopsy.[67, 68] 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.[69] Nonetheless, autoantibodies have been detected in many individuals suffering from long-term infection with *T. cruzi*.[70, 71] Meningoencephalitis can occur during the acute phase and is characterized by infiltrates of $CD8^+$ T cells.[72] Meningoencephalitis can also occur in patients with underlying HIV/AIDS.[73]

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.[74] With disease acquired through other means such as transfusion or transplant, the incubation period may be as long as several months.[75] 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 cases meningoencephalitis.[76, 77] The risk of severe disease and mortality during the acute disease tends to be the highest with orally acquired infection.[78]

In a few patients, but clearly only the minority, a characteristic hemi-facial edema or swelling called a chagoma develops at the site of the bite 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.[79, 80] It occurs mostly as a unilateral swelling. The swollen eyelid is firm to the touch, and there may be associated conjunctivitis. If the bite occurs elsewhere, the adjacent area is erythematous, brawny, 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. Patients at this point enter the chronic phase and, although they may remain asymptomatic, they are capable of transmitting 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 which may later manifest with injury to the heart or gastrointestinal system.

In as many as a third of patients, there is a progression of the disease that usually manifests as cardiac or gastrointestinal disease.[81, 82] There continues to be much controversy over the exact mechanism that leads to this delayed appearance of symptoms, but there is evidence that parasite persistence may be involved in the development of cardiac manifestations.[81] Another factor is the genetics of the parasite. There are six different 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 costameres in cardiomyocytes become disrupted during intracellular infection with the amastigote stage, and this is thought to make a major contribution to the cardiomyopathy so typically seen in the chronic infection.[83]

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 progression of cardiac disease may lead to death.[84] Some investigators believe that it is the conduction system disturbances that represent the most common cause of death from Chagasic 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.[85] The pathogenesis of gastrointestinal disease involves denervation due to destruction and fibrosis of the submucosal and myenteric nerve plexuses.[86] 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.[87]

Disease is not limited to the heart and gut. Rarely it also leads to megaureters, megabladder, mega-gallbladder, and bronchiectasis. Adult patients may experience reduced night vision.[88]

A reactivation of Chagas disease is now described in patients who become immunocompromised through infection with certain pathogens, such as HIV-1, or through immunosuppressive medications. Patients suffering from 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.[73,89] Patients in the chronic phase of infection that acquire HIV can experience a reactivation of *T. cruzi* resembling the acute phase of the disease.[90]

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.[91] More recent estimates place the vertical transmission rate at around 5%.[31] Transmission can also occur during chronic Chagas disease, as was documented in the United States in August of 2010.[92] Congenital infection can lead to acute disease or may progress to chronic disease in the absence of treatment.[93]

Diagnosis (see Clinical Appendix)

The approach to diagnosis is based on the stage of disease. In acute disease the level of parasites in the blood is high enough that trypomastigotes can be detected on examination of Giemsa-stained blood smears, and both a thin and thick smear should be ordered (Fig. 7.6).[94] The use of PCR has greatly facilitated

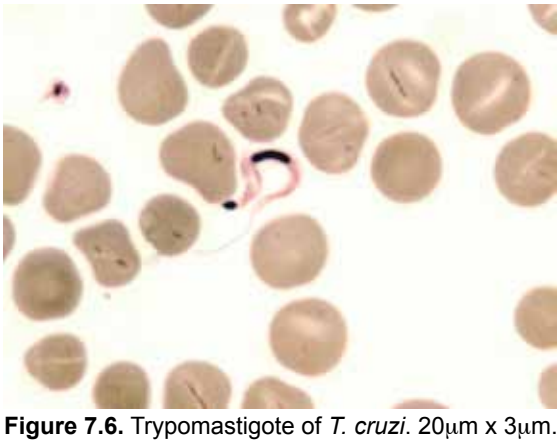


Figure 7.6. Trypomastigote of *T. cruzi*. 20μm x 3μm.

diagnosis in patients, allowing earlier detection with improved sensitivity over microscopic evaluation of blood smears.[94-96]

For chronic disease the diagnosis is usually based on the detection of serum IgG.[97] Since no currently available tests for Chagas serology have the required specificity, a diagnosis is based on two positive diagnostic serology tests.[98] It is of note that many patients present to their physician after a positive screening test result when they are donating blood. This screening test is not a diagnostic test 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 presents too much impracticality for most diagnostic facilities. Xenodiagnosis, employing uninfected kissing bugs, allowing them to feed on the patient, then dissecting the bugs some days later, can also reveal the presence of parasites in chronically infected individuals, but it is a special 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.[98-100] Both drugs are associated with high toxicity and incomplete cure rates in adults, especially when they are used to treat the chronic phase of the infection.[100] Benznidazole has been recommended for use in children who have either just acquired the disease (i.e., congenitally), or who are in the chronic phase of their infection.[101] Nifurtimox disrupts *T. cruzi* metabolism of carbohydrates, while benznidazole may promote lethal double-stranded DNA breaks in *T. cruzi*.[102, 103] 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 full treatment courses.

Additional sobering information suggests that once heart involvement begins, it may be too late for the drugs to have a clinical impact.[104] There is an urgent need to develop improved drugs and clinical algorithms. Newer drugs are being developed, some of which take advantage of known metabolic pathways.[105, 106] Another approach involves the development of a therapeutic vaccine to treat patients in their indeterminant phase in order to prevent or delay the onset of Chagas cardiomyopathy.[107] Such a vaccine would be potentially both cost-effective and cost-saving.[108]

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.[109, 110] In fact, the fourth recipient ever to receive a heart transplant suffered from chronic *T. cruzi* infection. Current immunosuppressive regimens have allowed for successful transplantation in a large number of patients that had developed cardiac manifestations of Chagas disease.[109, 110]

Despite a paucity of well-controlled trials on the efficacy of treatment for acute Chagas disease, there is a general consensus that treatment should be given for both acute and congenitally acquired disease.[111] Treatment decisions for chronic Chagas are based on 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 the age of 50 should be individualized based on risks and benefits. Treatment for patients with advanced cardiomyopathy or

gastrointestinal manifestations is currently not recommended. Unfortunately, these recommendations for 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.[104]

Prevention and Control

Control of Chagas disease depends upon interfering with two major routes of transmission: vector-borne and transfusion. Control of vectors, by prudent use of insecticides (pyrethroids), has significantly reduced transmission of *T. cruzi* in Brazil and Chile.[112, 113] Unfortunately, this trend has been slow to spread to neighboring countries and there is now evidence of insecticide resistance.[114] 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 the control of disease.[115] 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.[116] It is recommended that paid blood donors be banned in all countries in which Chagas disease is endemic.[115]

A more permanent solution, and one that interfaces well with the concepts of medical ecology, is building better housing for the poor.[117, 118] Houses constructed without a thatched roof, with slat board wood siding, or rough textured wall surfaces inside the house are relatively safe from kissing bug colonization. Keeping pet dogs and pigs out of the house further reduces the chances of acquiring Chagas disease.[119]

The risk to travelers is thought to be low, although there have been documented cases in travelers to endemic areas.[120] 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.[121] Consuming sugar cane juice or fruit drinks while on vacation on the beaches of Brazil, Central America or other parts of South America are likely low-risk activities despite the occasional outbreaks reported.[28-30]

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8. *Trichomonas vaginalis*
(Donné 1836)

Pronunciation: \tri-kə-ˈmō-nəs\ \vaj-ə-ˈnā-ləs\

Introduction

Trichomonas vaginalis (\trick-oh-MOAN-us\\vaj-gi-NAL-is\) is a flagellated, microaerophilic protozoan that is 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 is over 25% in some sexually transmitted infection clinics in the United States.[4-6] *T. vaginalis* infects both males and females. In males, infection can be asymptomatic and typically lasts 10 days. Most infected females are also asymptomatic, but infection can induce clinical disease that includes vaginal itching, inflammation, and purulent discharge with infection lasting for years if untreated.[7] Women infected with *T. vaginalis* typically experience periods of discomfort, and untreated infection may lead to infertility, pelvic inflammatory disease, cervical neoplasia, and increased susceptibility to HIV-1 infection.[8-12] *T. vaginalis* can also infect the newborn during their passage through the birth canal of an infected woman.[13] Infants may experience ectopic infection in the respiratory tract and other sites, as well. There are no reservoir hosts 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.[14] The genome of *T. vaginalis* was sequenced in 2007, and it is very large with approximately 60,000 protein-coding genes organized into 6 chromosomes.[15]

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.[16] Proof that *T. vaginalis* is indeed a pathogen came much later, in 1940, when 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 patients who were naturally infected.[17] This study also provided an accurate description of 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* has no true cyst form, but a pseudocyst form has been recognized that may play a role in cervical neoplasia.[18, 19] The trophozoite is motile, and possesses four flagella projecting from the anterior portion, an

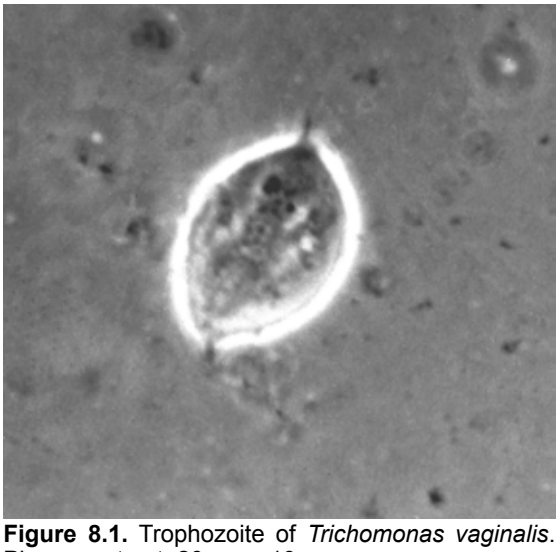
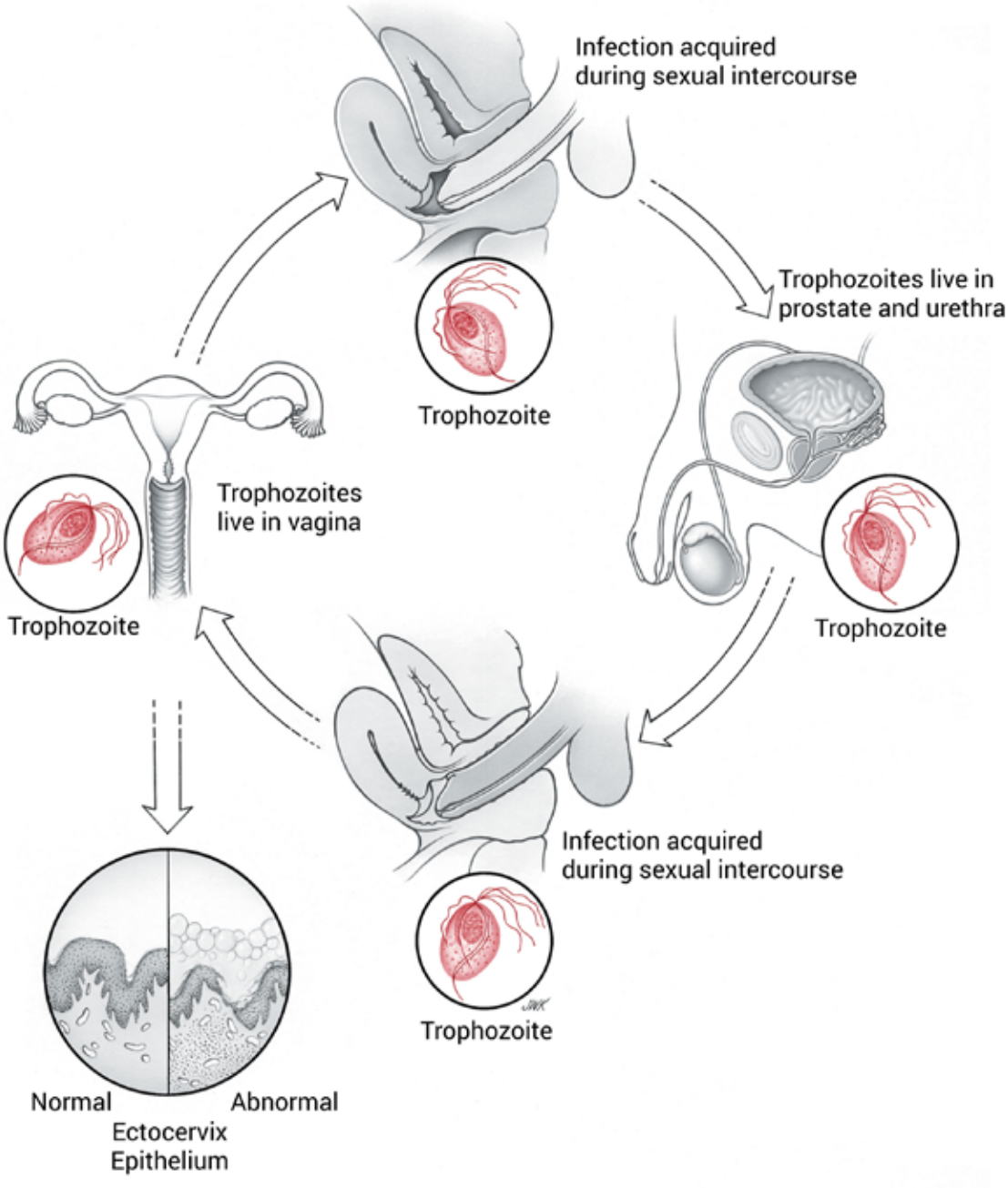


Figure 8.1. Trophozoite of *Trichomonas vaginalis*. Phase contrast. 20 μm x 10 μm.

Trichomonas vaginalis



undulating membrane formed by a posterior extending flagellum, and a rigid axostyle.[20] The axostyle is a sheet of microtubules believed to be involved in motility and mitosis.[21, 22]

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. In order to cause infection, the trophozoites must be able to adhere to epithelial cells. This is facilitated by adhesins and specific ligand-carbohydrate interactions.[23] Mannose and N-acetyl-glucosamine are two parasite membrane-associated sugar residues that are used for attachment.[24, 25] Secretion of lysosomal hydrolases, such as acid phosphatase, occurs at the host-parasite interface immediately following attachment.[26] These parasite enzymes are cytotoxic, causing the target cells to lyse, releasing their contents.[27] The cell debris is then ingested by the parasite. *T. vaginalis* phagocytizes epithelial cells, erythrocytes, and bacteria. The parasite's main 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.[28]

T. vaginalis reproduces by binary fission, and there is evidence for a sexual cycle, although no morphologically identifiable stage has been described.[29] 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, which functions in anaerobic metabolism.[30-34] The hydrogenosome contains some of the necessary enzymes for processing glucose to acetate to molecular hydrogen and putrescine biosynthesis.[35, 36] 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.[37]

Iron plays an important role in the parasite's attachment process.[23] Because *T. vaginalis* secretes proteases at the site of attachment, cell death is the usual outcome.[26] It is not known whether or not the release of molec-

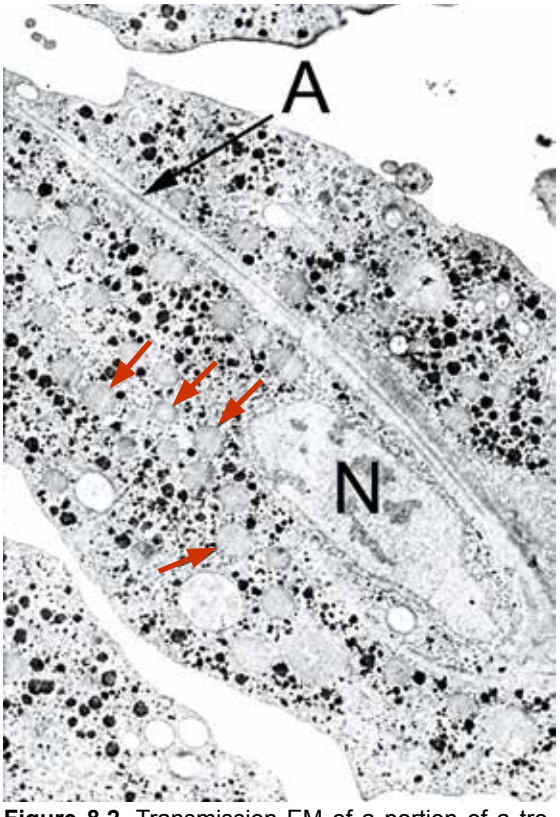


Figure 8.2. Transmission EM of a portion of a trophozoite of *T. vaginalis*. N= nucleus, A= axostyle. Red arrows indicate hydrogenosomes. Courtesy H. Shio.

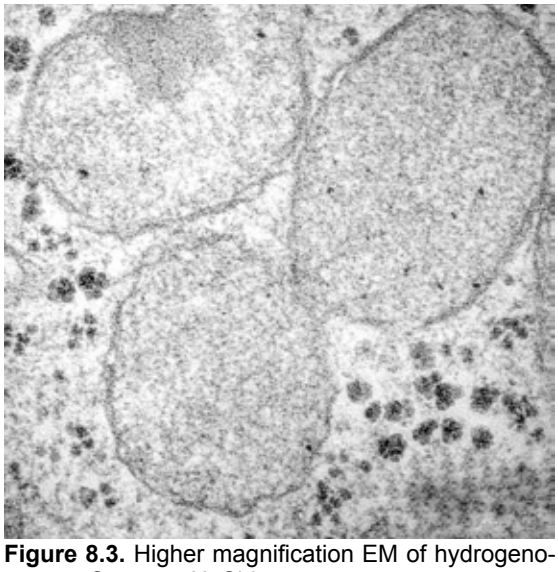


Figure 8.3. Higher magnification EM of hydrogenosomes. Courtesy H. Shio.

ular 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 with regards to their ability to induce damage in a mouse model, but the molecular basis for this variation is not fully understood.[38] *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 viruse (*Totiviridae*), as well as *Mycoplasma hominis*. In some areas, the majority of isolates show co-infection with these organisms.[39, 40] The relevance of these co-infections is currently the subject of active investigation.

Clinical Disease

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.[41, 42] Rarely, urticaria is a complication of heavy infection.[43] Infection with *T. vaginalis* typically raises the vaginal pH from 4.5 to greater than 5.[44] 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.[45] All of these signs and symptoms are exacerbated during menstruation.

Symptomatic disease in males can involve the urethra as well as the prostate. When the prostate becomes infected, pain in the groin and dysuria may be reported.[46] Infection increases the chances of transmission of HIV-1, in part due to disruption of the vaginal wall.[47, 48]

Infants born of mothers harboring the infection often acquire infection upon passing through the birth canal.[13] Clinical consequences of infection in newborns include urinary tract infection (females only), and rare involvement of the lung, resulting in a pneumonia-like syndrome.[49, 50]

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.[51, 52] Due to the high prevalence of asymptomatic infection, it is important to screen patients rather than rely on symptoms, as the majority of *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).[53] 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 approximately 65% decreasing with delay.[54] 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.[55] Nucleic acid probe testing is a highly sensitive option for diagnosis that has demonstrated efficacy for testing of vaginal swabs or urine.[55] Diagnosis by NAAT is, by far, more sensitive than any other method, and is now the preferred method in most hospital parasitology diagnostic laboratories.[53]

Treatment (see Clinical Appendix)

Metronidazole continues to be the drug of choice.[1] It can be given orally as a single 2 g dose but is often given as a 7-day course at 500 mg twice a day. Metronidazole is also available as a vaginal gel. Its use as an intravaginal suppository did poorly in a number of clinical trials.[45] The drug is typically well tolerated, but metallic taste, antabuse-like side effects with alcohol consumption and longer term treatment could have other toxicities.[56] 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 ferridoxin oxidoreductase and hydrogenase, deriving all their energy from glucose by alternate pathways.[57] 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), results in a significant reduction in reinfection but in high prevalence settings, reinfection is still seen.[58] 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

Use of 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, particularly when the number of sexual partners is low. Active screening is essential with the high rate of asymptomatic infections.

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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-ˈmō-dē-əm\

P. falciparum
(Welch 1898)

Pronunciation: \fal-ˈsi-pə-rəm\

P. vivax
(Grassi and Filetti 1889)

Pronunciation: \vī-ˌvaks\

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

Pronunciation: \ō-ˈva-lē\

P. malariae
(Laveran 1880)

Pronunciation: \məˈlerēˌē\

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\). Humans are commonly infected by four species of the parasite*: 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 certain areas of Southeast Asia with this species being responsible for a significant percentage of cases in certain regions.[1] Malaria remains the most important parasitic infection and one of the most prevalent infectious diseases of humans.

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

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] Most of these deaths were among children living in sub-Saharan Africa. Global morbidity and mortality from malaria are 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 for considering the technical and financial requirements critical to eliminating malaria as a leading cause of childhood deaths.[7]

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.[8, 9] 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.

Historical Information

Malaria afflicted humankind's ancestors as evidenced by its genetic footprint on the human genome.[10-12] 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 was able to differentiate quotidian, tertian, and quartan fevers and the clinical symptoms of the disease.[13] 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.

All concepts surrounding malaria changed within 20 years after Charles Laveran's 1880 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.[14] Laveran did not appreciate that there were different *Plasmodium* species and it was Camillo Golgi in 1886 who suggested there were at least two different forms of malaria.[15] 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.[16] Ultimately, Ross and Laveran were awarded Nobel prizes for their contributions.[17, 18]

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 blood.[19] While Miles Markus suggested in 1976 that a dormant stage of *Plasmodium* might exist and suggested the term 'hypnozoite', Wojciech Krotoski, an American physician, is credited with describing the dormant liver phase for *P. vivax* in 1980.[20-23]

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

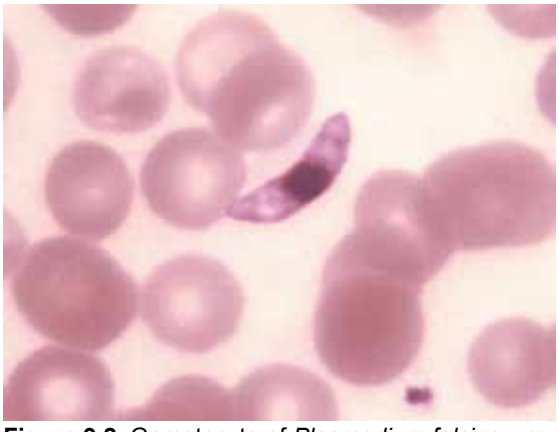
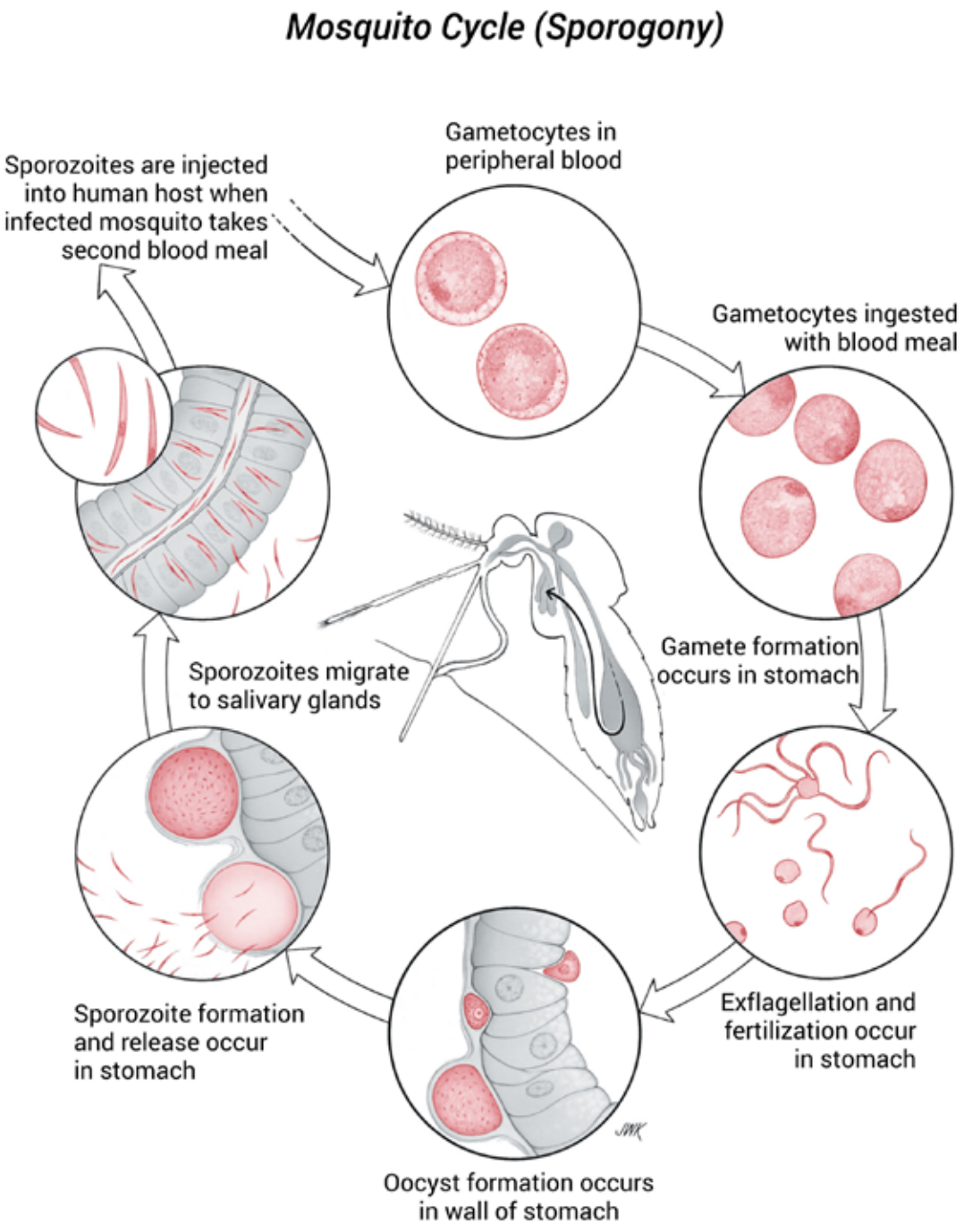


Figure 9.2. Gametocyte of *Plasmodium falciparum.*

parasite by nearly 300 years. The Peruvian bark of cinchona, or "fever tree", was first used during the early part of the seventeenth century, but the details of its discovery and its introduction into Europe are still controversial.[24-27] 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).[27]

The Greeks and Romans practiced the earliest forms of malaria control, albeit inadvertently, by draining swamps and marshes. Their primary purpose was reclamation of land. These techniques were continued for centuries before the role of the mosquito as 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 Orga-


Mosquito Cycle (Sporogony)
Gametocytes in peripheral blood
Sporozoites are injected into human host when infected mosquito takes second blood meal
Gametocytes ingested with blood meal
Sporozoites migrate to salivary glands
Gamete formation occurs in stomach
Sporozoite formation and release occur in stomach
Exflagellation and fertilization occur in stomach
Oocyst formation occurs in wall of stomach

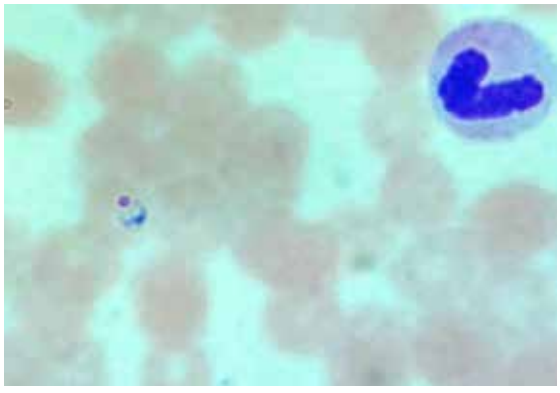


Figure 9.3. Signet ring stage of *Plasmodium* spp.

nization 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 the human malarias, and accounts for most of the mortality from the illness. *P falciparum* is the most prevalent of the human malarial infections and is mostly confined to tropical and subtropical regions. It is the primary cause of malaria in sub-Saharan Africa.

Identification of *P. falciparum* is usually based on the presence of 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 as long as 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 are unable to re-infect the liver. Recrudescence (reappearance of infected erythrocytes from the deep tissues into the peripheral blood) is common and can recur for about two years.

Plasmodium vivax

P. vivax infection is called benign tertian or vivax malaria. Red blood cells infected with *P. vivax* (Fig. 9.4, 9.17) are enlarged and, when properly 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 characteristic. Gametocytes appear simultaneously with the first asexual parasites. The duration of the viability of the sexual stages appears to be less than 12 hours. *P. vivax* produces the classic relapsing malaria, initiated

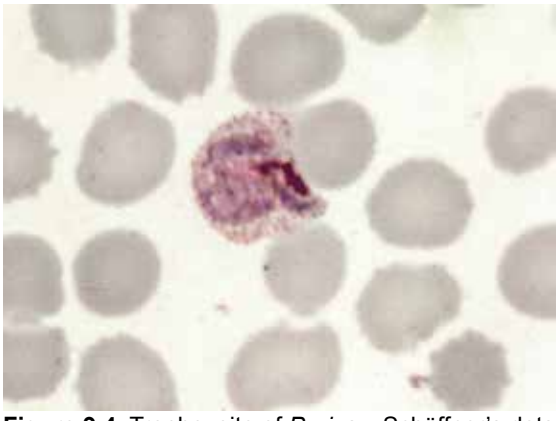


Figure 9.4. Trophozoite of *P. vivax*. Schüffner's dots on the surface of the infected red blood cell. The surrounding red cells are smaller than the infected one.

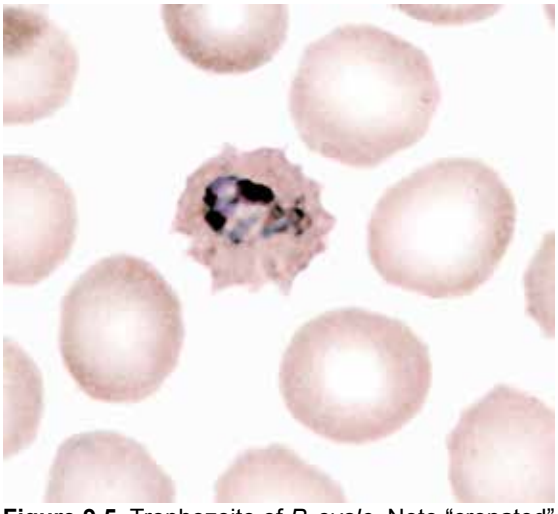


Figure 9.5. Trophozoite of *P. ovale*. Note "crenated" appearance of infected red cell. Courtesy M. Guelpe.

from hypnozoites in the liver after a period of latency. Relapses can occur at periods ranging from 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 to discrete areas of the Western Pacific. *Ovale* malaria produces a tertian fever clinically similar to that of 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).[28] *P. ovale wallikeri* appears to have a shorter latency period than *P. ovale curtisi*.[29]

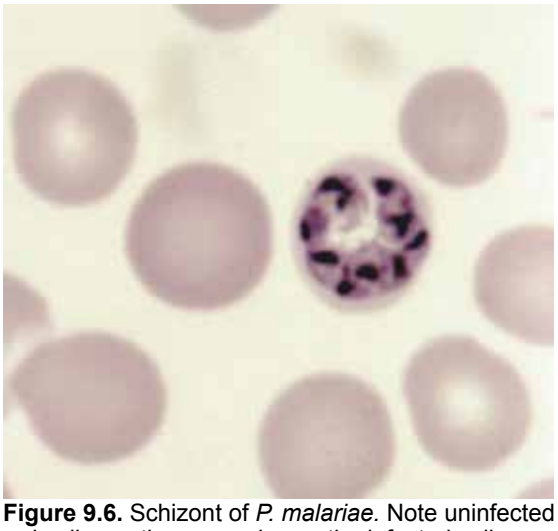


Figure 9.6. Schizont of *P. malariae*. Note uninfected red cells are the same size as the infected cell.

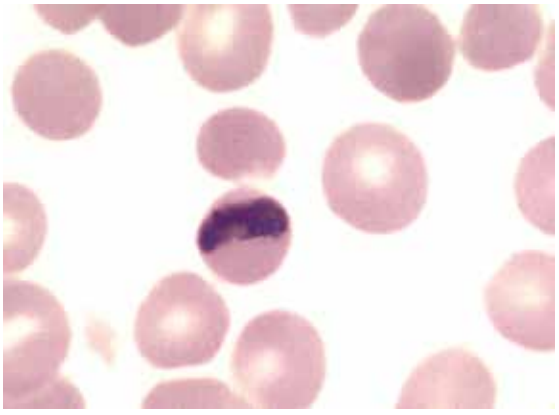


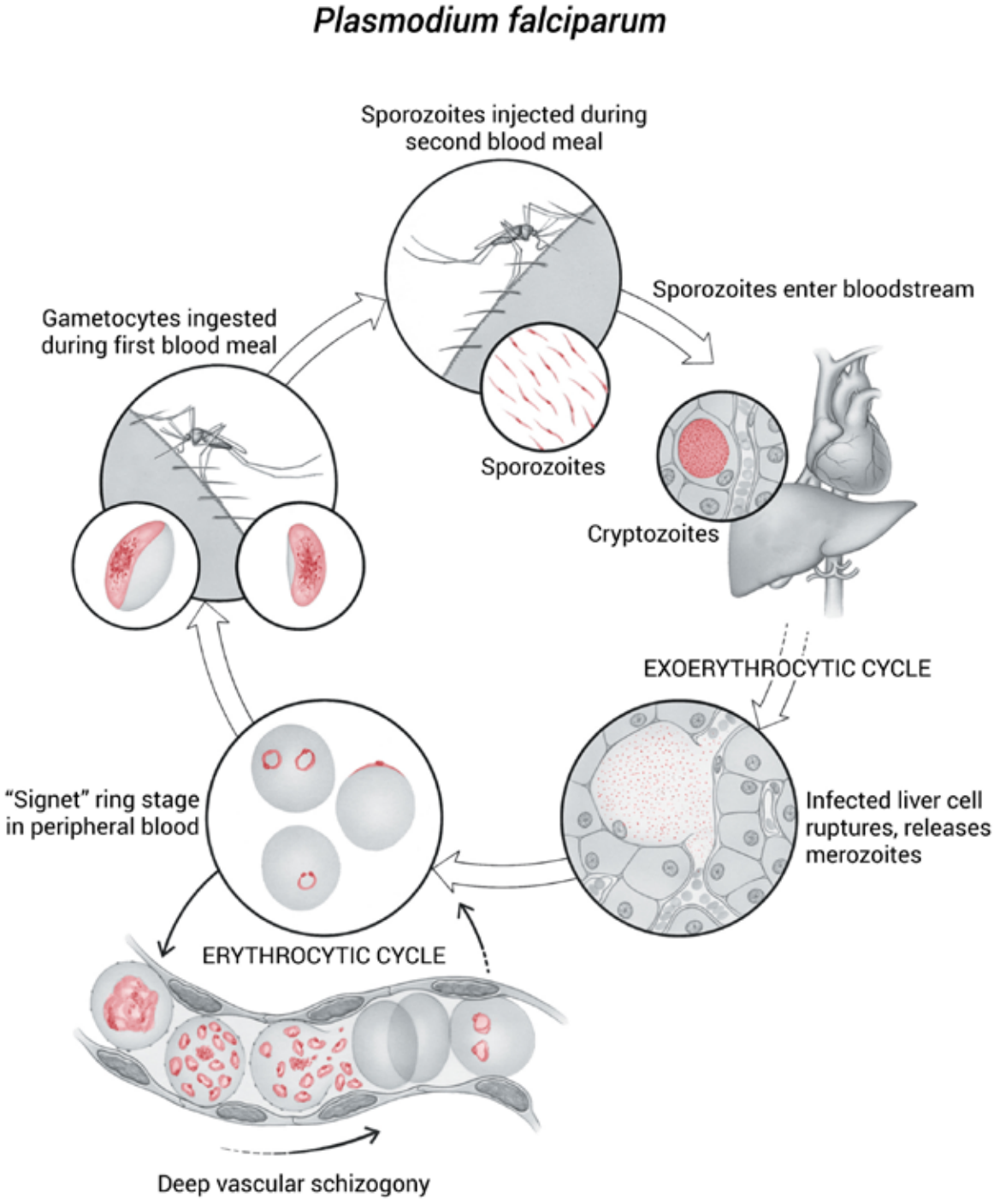
Figure 9.7. *Plasmodium malariae* trophozoite.

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 infection in humans is not as intense as those caused by the other *Plasmodium* species. Most current evidence indicates that *P. malariae* does not relapse. It does recrudesce due to chronic low-level parasitemia that may persist for decades.[30, 31] 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 monkeys occasionally infect humans.[18] In 1932, Knowles and Das Gupta described the experimental transmission of *P. knowlesi* to humans.[32] It later became established as the fifth human malaria.[33, 34] *P. knowlesi* was thought to be restricted to limited areas of Southeast Asia but cases are being diag-


Plasmodium falciparum
Sporozoites injected during second blood meal
Sporozoites enter bloodstream
Gametocytes ingested during first blood meal
Sporozoites
Cryptozoites
EXOERYTHROCYTIC CYCLE
"Signet" ring stage in peripheral blood
Infected liver cell ruptures, releases merozoites
ERYTHROCYTIC CYCLE
Deep vascular schizogony

Plasmodium vivax

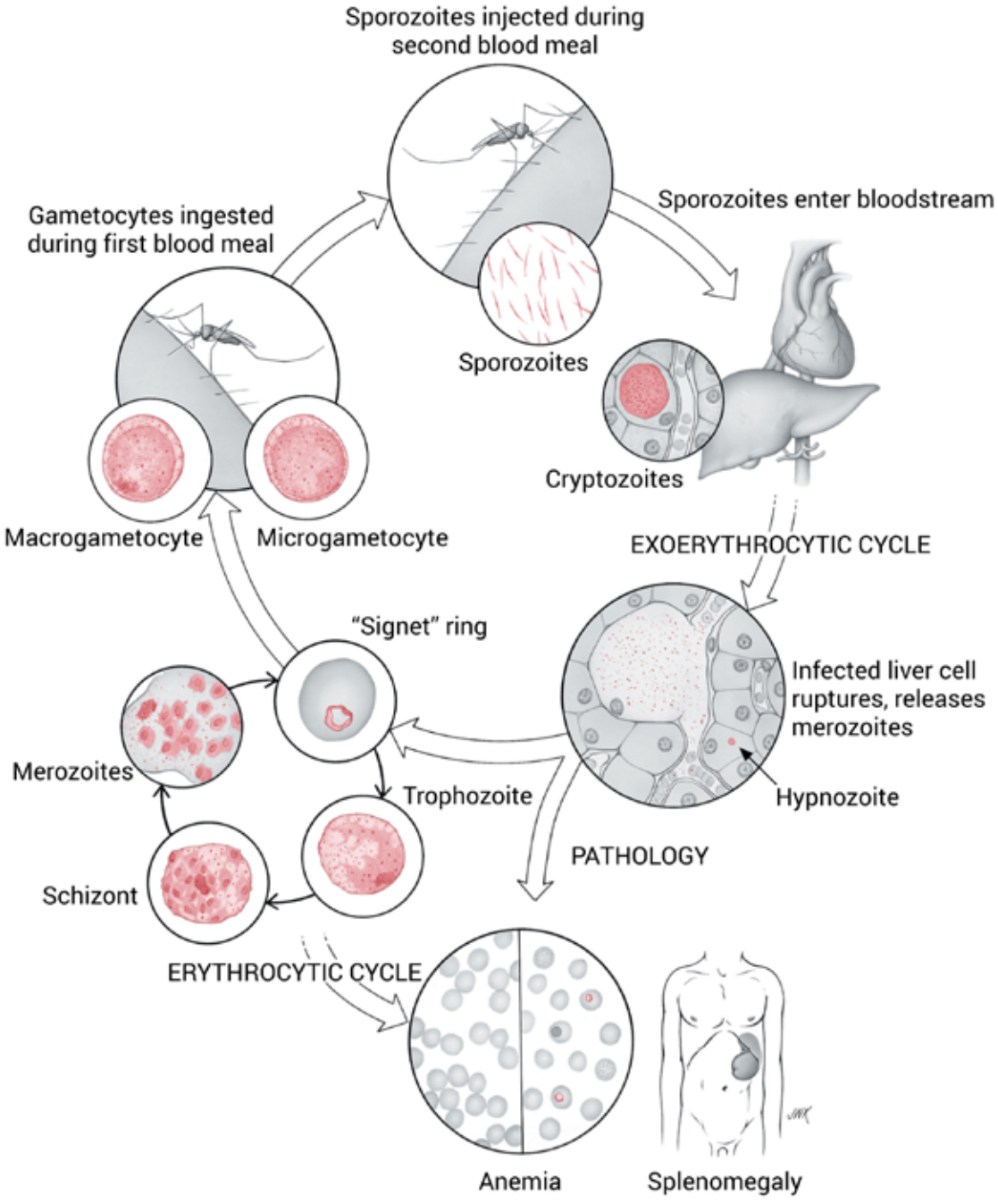


Figure 9.8. Sporozoites of malaria in infected mosquito stomach preparation.

nosed in more areas of Southeast Asia, either through increased awareness or an expansion of the geographic range.[1, 35] 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.[36-38] The disease it causes can range from mild to severe. Most notable is the quotidian fever (24-hour cycle). Certain other simian malarias such as *P. cynomolgi, P. brasilianum*, *P. eylesi, P. inui*, *P. schwetzi,* and *P. simium* may be transmissible to humans through the bite of a mosquito.[39-41]

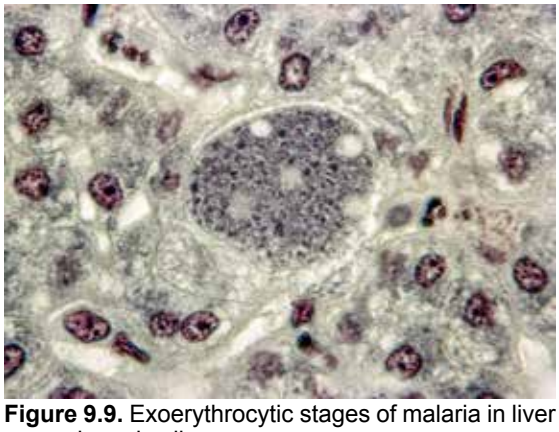


Figure 9.9. Exoerythrocytic stages of malaria in liver parenchymal cell.

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.[42] These are cleared from the circulation within an hour, and eventually reach parenchymal cells of the liver. Based on animal models the sporozoites may spend only about 10 minutes in the area of inoculation and then only about 2 minutes in the circulation before they are cleared or invade hepatocytes.[43] How sporozoites traffic to and enter the liver has been a subject of much investigation.[44-46] Evidence suggests that sporozoites bind to CD68 on the surface of Kupffer cells facilitating entry into hepatocytes.[47] Once inside the liver cell, the parasites undergo asexual division (exoerythrocytic schizogony; Fig. 9.9). A prescribed number of merozoites are produced over a period of 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 9 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.[48]

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. By definition, a parasitologic malarial relapse is the reappearance of parasitemia in peripheral blood in a sporozoite-induced infection, following adequate blood schizonticidal therapy.[49] It had been long accepted that the exoerythrocytic forms of relapsing malaria persist in the liver as a result of cyclic development (rupture of infected cells and invasion of new cells).[50] 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.[51]

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, eradication of parasites from the peripheral circulation with drugs aborts the acute infection. Subsequently, a fresh wave of exoerythrocytic merozoites from the liver can reinitiate the infection. The dormant parasites, or hypnozoites, can remain quiescent in the liver for as long as five years.[31, 52-54] To achieve radical cure, it is necessary to destroy not only the circulating parasites but also the hypnozoites.[55]

P. falciparum and *P. malariae* do not develop hypnozoites and therefore lack the capacity to relapse. Thus, for infections with *P. falciparum* and *P. malariae*, drugs that only eradicate parasites in the peripheral circulation are sufficient to achieve cure. Untreated *P. falciparum* can recrudesce for 1–2 years through the continuation of the erythrocytic cycle, which for periods of time remains at a subclinical, asymptomatic level. *P. malariae* can similarly recrudesce for 30 years or more.[30]

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 of a complex sequence of events, beginning with contact between a free-floating merozoite and the red blood cell.[56] Attachment of the merozoite to the erythrocyte membrane involves interaction between *Plasmodium* binding proteins

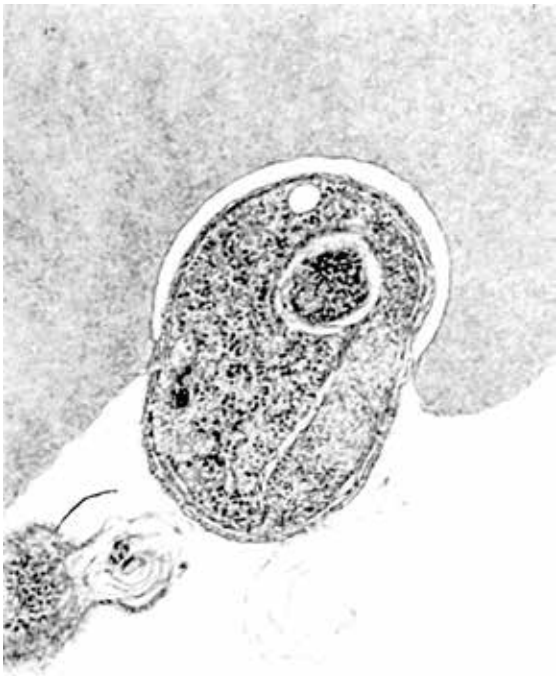


Figure 9.10. Transmission EM of a merozoite entering a red cell. Note points of attachment. Courtesy S. Langreth.

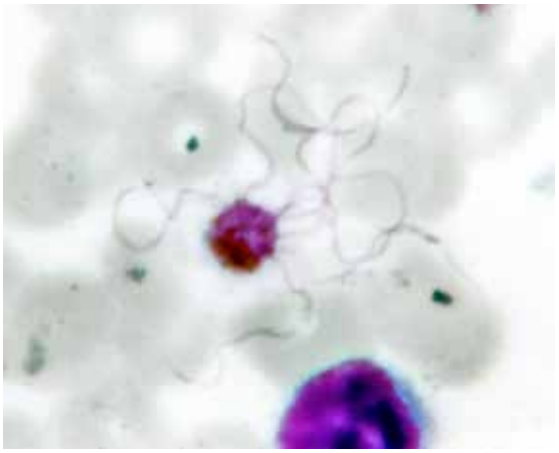


Figure 9.11. Exflagellation of the microgametocyte of a malaria parasite. Each "flagella" is actually a male gamete.

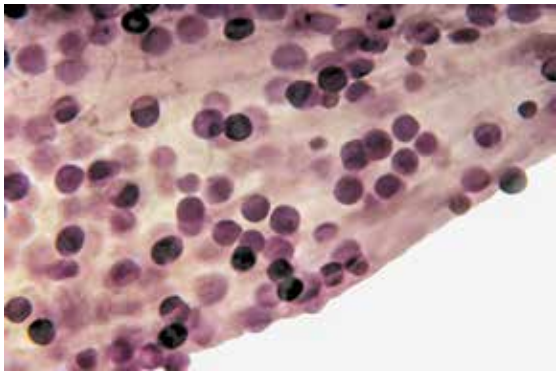


Figure 9.12. Portion of an infected mosquito stomach. Note numerous oocysts on outer wall.

and specific receptors on the surface of erythrocytes.[57] As an example, transferrin receptor 1 appears to be the receptor for *P. vivax* reticulocyte binding protein 2b.[58] Thereafter 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.[59]

Once within the cell, the parasite begins to grow, first 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

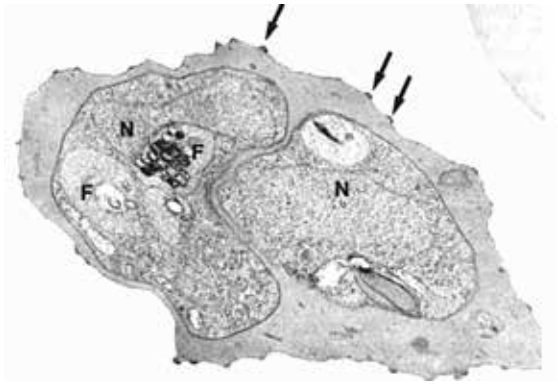


Figure 9.13. Transmission EM of red cell infected with *P. falciparum*. Arrows indicate ("knobs") points of attachment to host endothelial cells. N=nucleus, F=food vacuole. Courtesy S. Langreth.

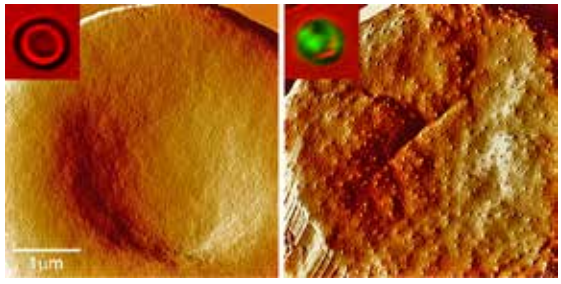
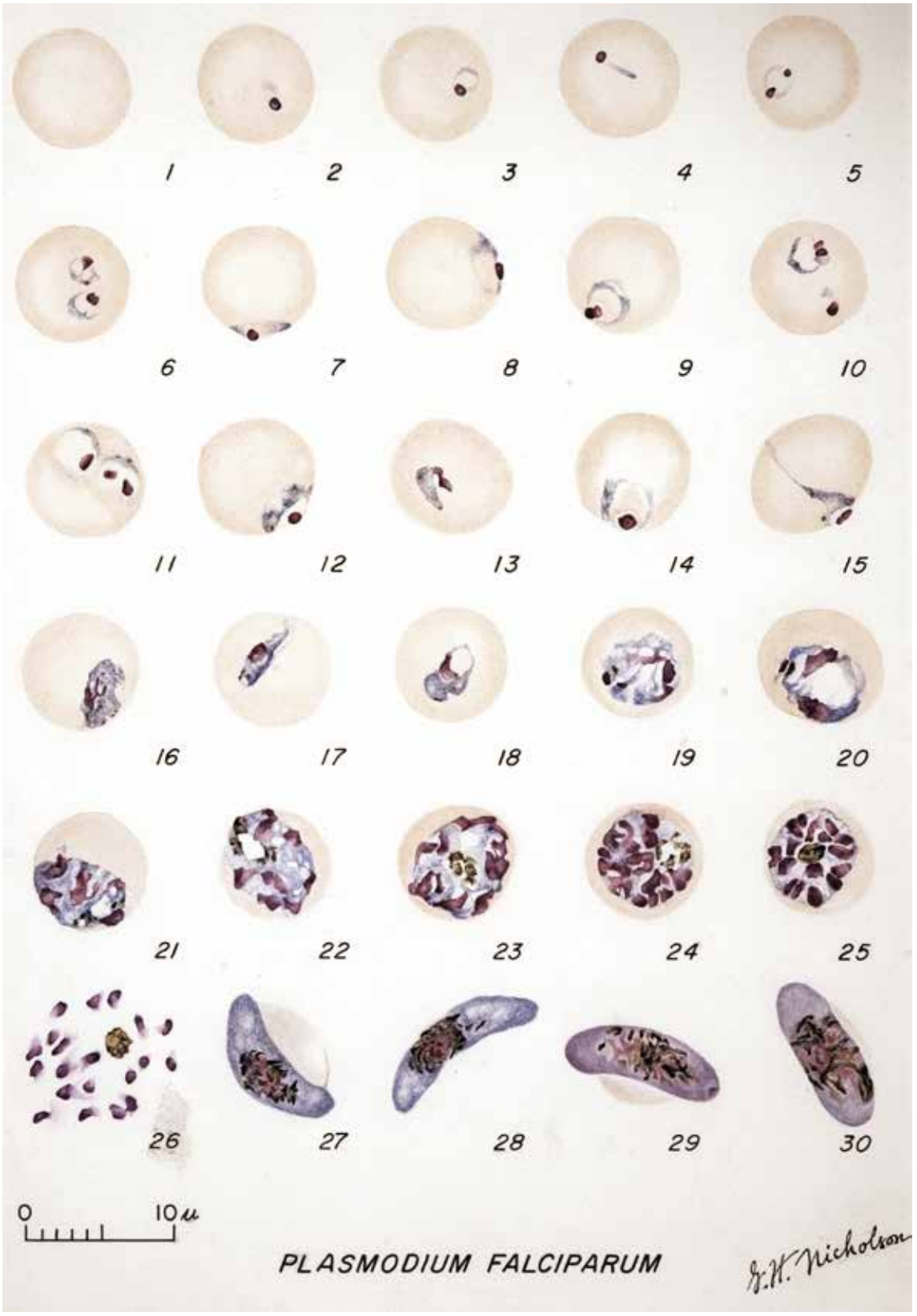


Figure 9.14. Atomic force microscopy of normal (left) and *Plasmodium falciparum*-infected (right) red cells. Courtesy J. Dvorak.

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.[46]

The asexual cycle is 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 for completion of 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

Figure 9.15. Child infected with malaria, probably *P. malariae*. Note enlarged spleen.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PLASMODIUM FALCIPARUM
J. H. Nicholson

Figure 9.16.

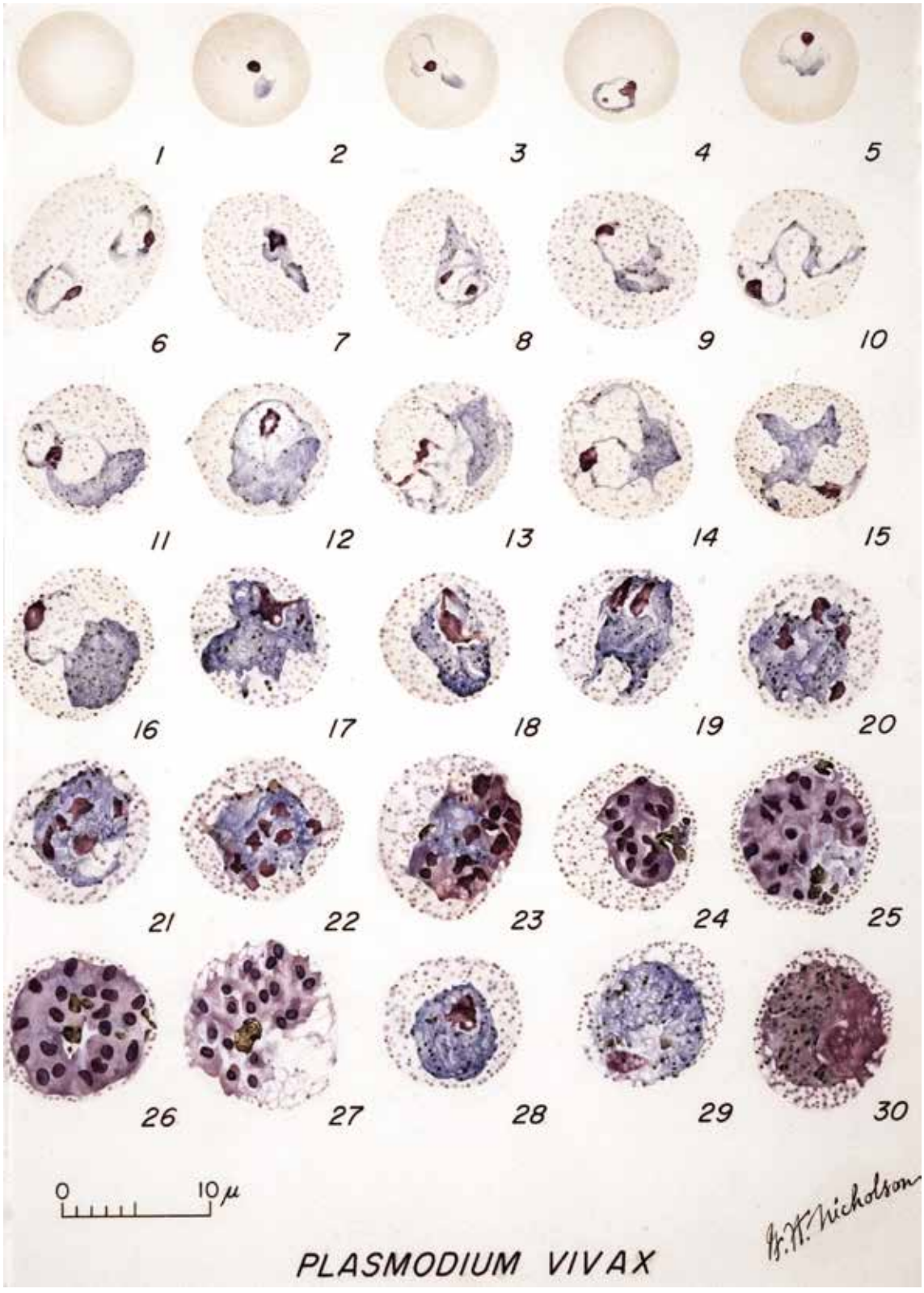
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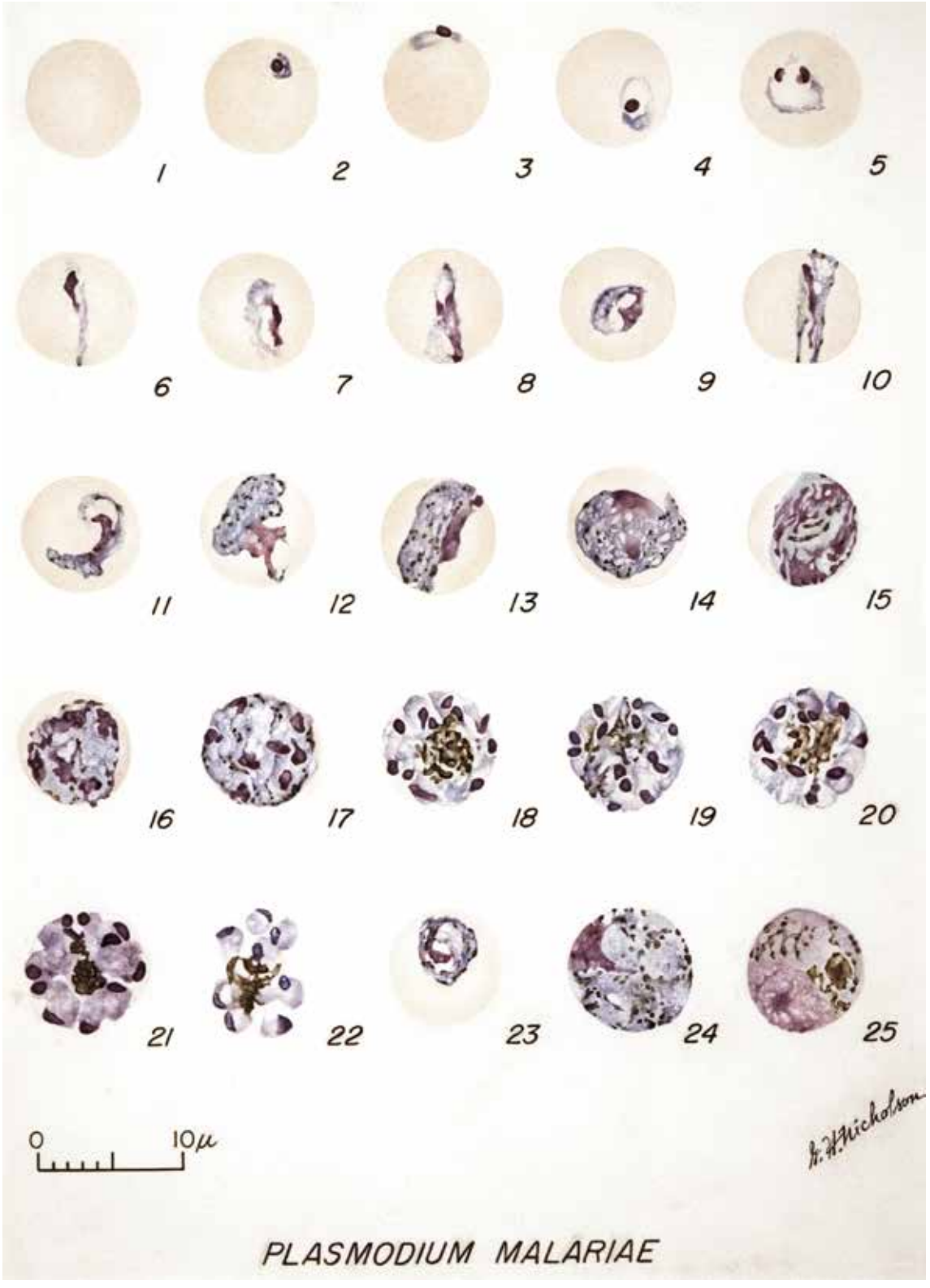
Figure 9.17.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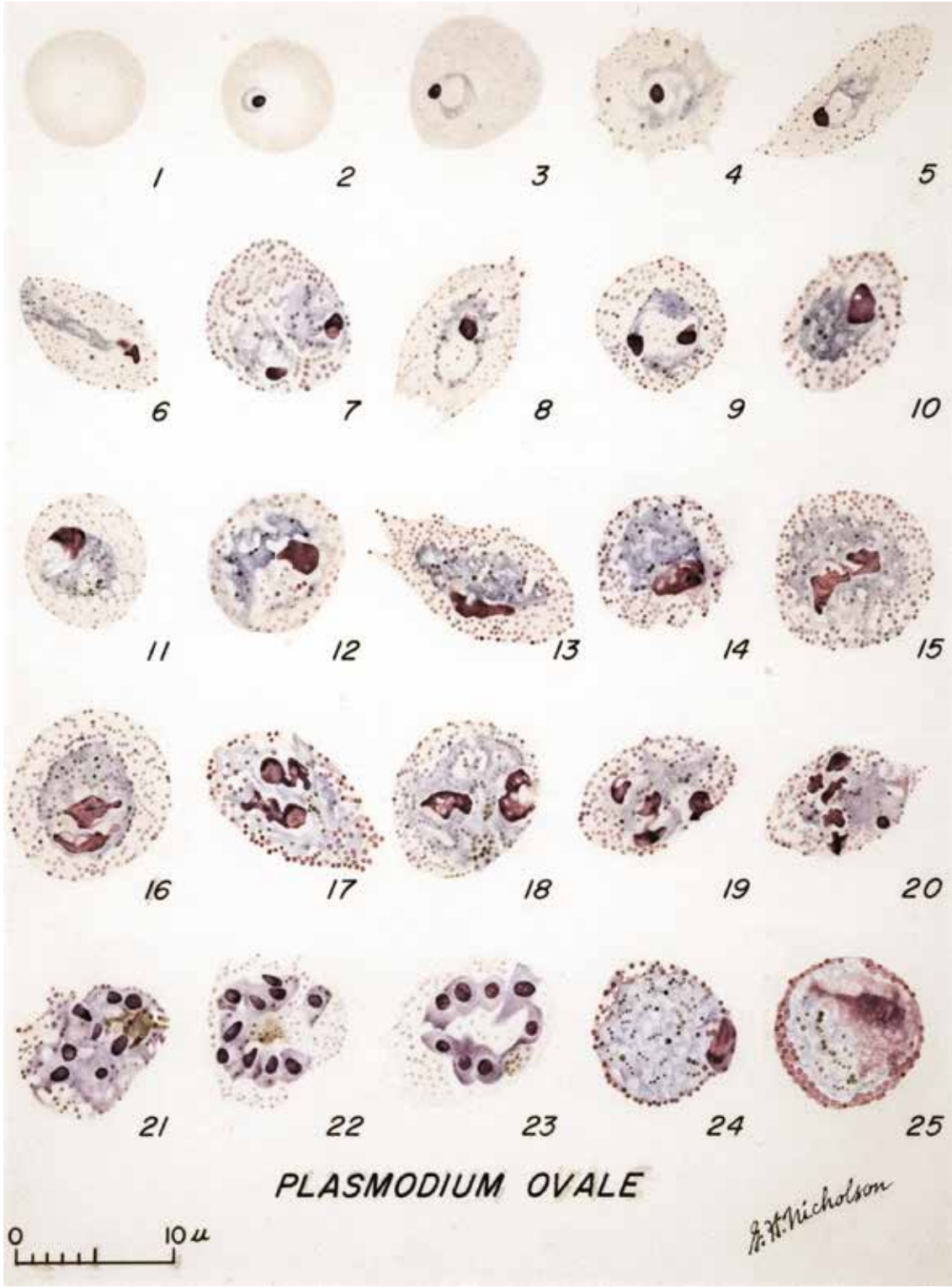
PLASMODIUM MALARIAE

Figure 9.18.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F. H. Nicholson

Figure 9.19.

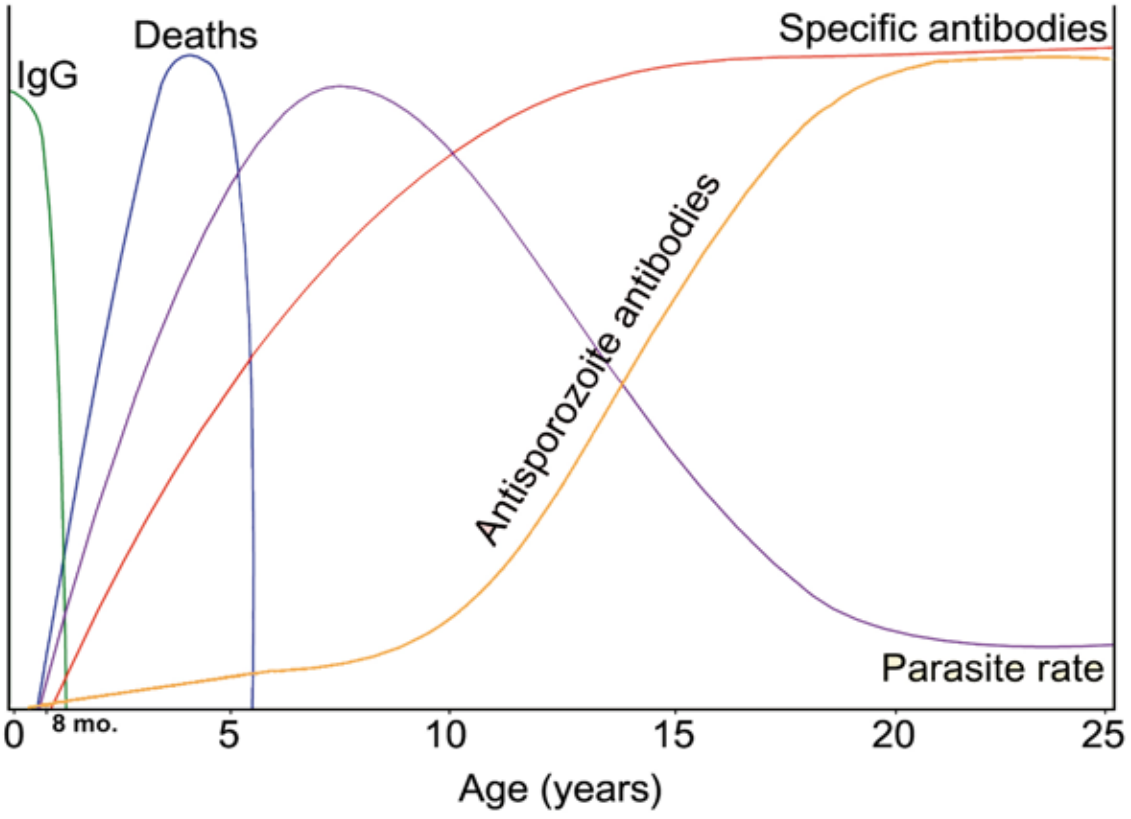

Figure 9.20. Graph indicating relationships between age of patient, susceptibility to infection, production of antibodies against different stages of parasite, and lethality of infection.

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 and thus the term quartan fever is applied.[15] The characteristic periodicity of the fever, based on synchronous infections, is not invariable; the early phases of infections are often not synchronous. Some infections may be due to two or more broods of parasites, with the periodicity of one independent of that of the others.

Infection with erythrocytic phase merozoites 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.[60] 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 as a result of transplacental infection is perhaps more common than originally suspected, due to its high rate of spontaneous clearance, but appears to be increased in the context of HIV infection.[61-65]

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 elon-

gate into diploid vermiform ookinetes (\ō-ə-ˈkī-ˌnēts\), which penetrate the gut wall and come to lie under the basement membrane (Fig. 9.12). The parasites then transform into oocysts (\ˈō-ə-ˌsists\) within 24 hours of ingestion of the blood meal. Development of sporozoites follows, leading to the production of 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 major differences in the pathologic course they pursue, they are most simply differentiated on the basis of 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 methods for malaria parasite detection and characterization are becoming increasingly sensitive, and are supplanting microscopy in more advanced laboratories (see Diagnosis).[66]

Cellular and Molecular Pathogenesis

The release of cytokines following rupture of infected erythrocytes induces fever and the consequent chills and sweating associated with malaria.[67] The pathogenesis of general malaise, myalgia, and headache appear to be related to the release of certain cytokines and their levels correlate with disease severity.[68]

Cerebral malaria is the most devastating manifestation of severe *P. falciparum* infection.[69] Cerebral malaria is caused by a complex process involving sequestration of erythrocytes as well as cytokine release and endothelial activation.[70, 71] 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.[72, 73] 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).[74, 75] Although not essential for cytoadherence, the knobs seem to enhance binding.[76] These knobs are induced by the parasite and facilitate endothelial cell binding by the infected erythrocytes to a number of endothelial targets.[77, 78] 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 whilst CD36 is likely the most important in other organs.[48] Cytoadherence in the placentae of women in their first pregnancies involves parasite binding to CSA.[79, 80] Sequestration of malaria parasites in the placentae of primigravid females is a major cause of death, fetal mortality, fetal wastage and low birth weight.[80-82]

Malaria can also be responsible for 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 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.[83] Disseminated intravascular coagulopathy occurs in

severely infected individuals.[84]

The spleen plays a major role in host defense against malaria (Fig. 9.15). Parasitized cells accumulate in its capillaries and sinusoids, causing general congestion. 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.[85] 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.[86] The liver is darkened by the accumulated malarial pigment 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.[87] Blackwater fever is a consequence of severe hemolysis exacerbated by the host 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 by endothelial proliferation, apparently caused by the deposition of immune complexes.[15] 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, but it is also possible that the parasites can infect the fetus through an intact placenta or at the time of birth.[64]

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, but the competing hypothesis is that it is the chronic immune activation that drives Burkitt's lymphoma.[88-90]

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.[91] In Africa as well as many other parts of the tropics, HIV and malaria overlap in their geographic ranges and the populations they infect.[6, 92] The focus is often on how HIV infection predisposes individuals to an increase in 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.[93-95] There is also evidence to suggest a relationship between HIV-induced reduction of $CD4^+$ T cell counts and a rise in incidence of malaria.[95-99] HIV does confer an increased susceptibility to symptomatic malaria.[100, 101] There is conflicting information regarding the severity of clinical malaria when it manifests in HIV-infected individuals, but most evidence suggests that they experience higher levels of parasitemia and an increased risk for severe malaria.[102, 103]

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.[48, 66] 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, although 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 chemoprophylaxis may not develop any 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 in approximately 40% of children with severe malaria.[104] Initially, there can be mild anemia with an elevation of the reticulocyte count. The leukocyte count tends to be normal or even low.[105] The eosinophil count is characteristically low (eosinopenia) in acute malaria and a robust rise in eosinophils and hemoglobin after completing malaria therapy is usually seen in successfully treated patients experiencing a good recovery.[106]

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 spontaneous cure of *P. falciparum* malaria within two years and of *P. vivax* and *P. ovale* malarias within five years, although 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 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.[15, 107] 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 who are intravenous drug users may signal the presence of induced malaria. Induced malaria is malaria that results from direct transfer of infectious merozoites from the blood of an infected individual to a new host. Hypnozoites do not develop with 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 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. There are a number of genetic factors in human populations that confer varying levels of susceptibility to malaria.[46, 108, 109] 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.[108-110] 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% and is an excellent example of a balanced polymorphism.[111]

In 2011, a group of researchers demonstrated that sickle hemoglobin provides protection against malaria through induction of the expression of heme oxygenase-1, which prevents accumulation of circulating heme.[112] This is particularly protective against cerebral malaria. Sickle hemoglobin is also able to inhibit the activation and expansion of $CD8^+$ T cells, resulting in lower cellular activation and higher cellular reactivity in response to malarial antigens.[112-114] Hemoglobin C mutations appear to provide similar protection against *P. falciparum* malaria in certain ethnic groups.[115, 116]

Glucose-6-phosphate dehydrogenase (G6PD) deficiency, β-thalassemia, and ovalocytosis, the latter common in Southeast Asia, have been implicated as mediators of innate resistance against *P. falciparum* infection.[117] It has been suggested that the protective effect of thalassemia may be related to enhanced immune recognition and clearance of parasitized erythrocytes.[118] Evidence also supports the idea that certain of these hemoglobinopathies reduce the intraerythrocytic multiplication of *Plasmodium* species.[119]

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 itself is the actual receptor, most West Africans are negative for the Duffy blood type and have decreased susceptibility to infection with *P. vivax*.[111, 120] Instead, they have a higher than normal incidence of infection with *P. ovale*.[121]

Acquired immunity (incomplete nonsterile) develops after long exposure to malaria and is characterized by low levels of parasitemia. Immune individuals have intermittent parasitemia with only mild symptoms. This clinical state has been referred to as premunition, in contrast with classic immunity, which prevents any degree of infection. Premunition does not last for life and individuals returning to endemic areas after even only one year away often have lost this protective immunity.[122-124]

Diagnosis (see Clinical Appendix)

For over a century a definitive diagnosis of malaria 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 makes possible the identification of the species of malaria and an indication of the level of parasitemia in the infected host.[125] Under normal conditions, both thick and thin smears should be examined. When malaria is suspected, the clinician should take a thorough travel history. Should the initial blood smears prove to be negative, new specimens should be drawn at 6-hour intervals.

Identification of 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 not identified.[126] 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.[127] These rapid tests detect the presence or absence of PfHRP2, which is present in *P. falciparum*, and then allow detection of other species and species level discrimination by using lactate dehydrogenase or aldolase antigens that are species-specific.[128-130] Unfortunately a number of rapid diagnostic tests can have poor sensitivity and specificity for the diagnosis of *P. knowlesi*.[131] A number of NAATs have been developed that have been employed in research and epidemiological studies.[132] The introduction of portable platforms (e.g., loop-mediated isothermal amplification (LAMP) assays) may allow molecular testing to move from the research setting to field diagnosis.[133-135]

The identification of 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 and in use in the field for pyrimethamine, sulfadoxine, cycloguanil, chloroquine, and artemisinin.[136-140] 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.[141] 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 risk of infection or assessment of the effects of intervention strategies for the control of malaria.

Treatment (see Clinical Appendix)

The long reliance on chloroquine to treat *P. falciparum* is no longer tenable, due to the worldwide spread of drug resistance.[142] The two major choices for treatment of 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.[143, 144] Artesunate treatment has demonstrated a significant reduction in mortality in both adults and children compared to treatment with quinine.[143, 144] While artemisinin-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.[145, 146]

A significant and striking exception to this worldwide recommendation had been malaria treatment in the United States. For parts of the world where artesunate is not available, 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.[147] Quinidine gluconate remained the only approved therapy by the FDA for the parenteral therapy of severe malaria until 2019 when it was announced that intravenous artesunate would become available in the U. S. for the treatment of severe malaria. Intravenous artesunate should be considered early in therapy given the favorable comparative data.[148] 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 a number of important 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 tracking of blood glucose; cautious fluid management, and delayed initiation of enteral feeding; factors critical to successful treatment outcomes.[48] Seizures may need to be managed with anti-seizure medications or benzodiazepines, and protection of the airway may be required in severe cases. Broad-spectrum intravenous antibiotics may be indicated in certain cases due to the high rate of bacterial sepsis that can occur in severe malaria.[149]

Exchange transfusions, once felt to be essential in management of severe malaria, are no longer recommended by the CDC, since a meta-analysis of previous studies revealed no significant survival advantage.[150] Blood transfusions for patients have been recommended based on the results of small randomized trials and a few large observational trials. Transfusion is recommended if hemoglobin levels are below 4 g/dL in all cases, or if hemoglobin levels are below 6 g/dL and there is parasitemia greater than 20%, or other concerning clinical features.[151, 152]

In contrast to severe or complicated malaria, non-complicated malaria is defined as symptomatic malaria with lack of any of the defined 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. For the treatment of uncomplicated malaria, if a patient has *P. falciparum* infection acquired from a region without chloroquine resistance, such as the Caribbean and Central America west of the Panama Canal, chloroquine can be used.[153] 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 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.[154] There are several available forms of artemisinin combination therapy with no one choice clearly superior over the other.[154]

Alternative drugs may be selected on a region-by-region basis with knowledge of local resistance rates.[155] 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.[156-158] 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.[159] Malarone (atovaquone-proguanil) is an effective option that has demonstrated superior efficacy over mefloquine treatment and may have a better safety profile.[160, 161] 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.[162]

When the infecting strain is *P. vivax* or *P. ovale,* treatment may not resolve infection by the hypnozoite liver stage, these must be cleared with a course of primaquine to prevent relapses. 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.

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.[153] 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 are hesitant to prescribe or take mefloquine due to concerns regarding its potential to induce psychosis.[163] 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 prior to using primaquine due to the danger of hemolysis.[164]

Prevention and Control

There are more than 300 species of *Anopheles* mosquitoes, but only about 60 species are considered important vectors of malaria. Some of the 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 important 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 of the imported variety. 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 largest number of imported cases. In addition, there is a steady incidence of malaria among travelers returning from endemic areas. 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. Outbreaks of *P. vivax* in southern California have been associated with the vector species, *An. hermsi*.[165] There are rare reports of malaria transmission in the United States, usually limited to one or two cases transmitted by local anopheline mosquitoes.

In addition to chemoprophylaxis when indicated, 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, but not without issues of proper use.[166] Clothing that covers much of the skin and insect repellents, particularly those containing DEET or picaridin, are useful adjuncts to transmission prevention.

Controlling the mosquito vector remains the most practical method for wide-scale control of malaria. 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 of interrupting transmission by targeting only those infected mosquitoes coming to feed in

houses. The rising costs of these insecticides and the development of resistance by the insects have severely limited their application and usefulness. Insecticide-impregnated bed nets have been shown to have a significant impact on the morbidity and mortality of infection due to *P. falciparum* and *P. vivax* in China, and to *P. falciparum* in Africa.[167, 168] Malaria control schemes based on genetic modification of the capacity of vector mosquitoes to transmit the parasite have been suggested.[169] In addition, the development of more efficient methods for introducing advantageous genes into the mosquito genome are being investigated, as well as methods for replacing vector populations in the field with populations of mosquitoes unable to transmit the parasite.[170, 171]

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 the development of methods for the *in vitro* cultivation of the asexual and sexual stages of *P. falciparum* by Trager and Jensen.[172] The revolution of molecular biology made possible the identification of specific genes coding for specific antigens and sub-unit vaccines became possible.[173]

There are three phases of the malaria life cycle that have been targeted by the vaccine investigators.[174, 175] 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. Secondarily 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 useful 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 useful in areas of low transmission, or in children and women of childbearing age in areas of high transmission.

Vaccines directed against the erythrocytic (blood) stages of the parasite are not expected to induce sterile immunity and totally prevent infection. Rather, it is expected that a successful vaccine could reduce the parasite burden, eliminate most deaths and reduce 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 could interrupt transmission to additional victims. In combination 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. The investigational malaria vaccines are listed by stage of clinical development at The Malaria Vaccine Initiative website (www.malariavaccine.org). The most advanced candidate provided modest protection in children hence the need to continue to evaluate the earlier stage vaccines in the pipeline.[176]

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

(Tyzzer 1929)

Cryptosporidium parvum

Pronunciation: \krip-tō-spȯr-ˈi-dē-əm\ \pahr'vŭm\

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 throughout the world, 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 on a regular basis: *C. parvum* (\PAAR-vum\), *C. hominis*, *C. meleagridis*, *C. felis*, *C. canis*, *C. muris*, and *Cryptosporidium* pig and deer species.[4-10] The majority of human infections are caused by *C. parvum and C. hominis*, which also infect sheep, cattle, birds, rodents, and non-human primates.[11] This chapter will concentrate on *C. parvum,* with the assumption that disease in humans caused by other related species results in a similar clinical picture.

In 1993, the city of 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 suffering from 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 genome of *C. hominis and C. parvum* have been sequenced.[11, 16, 17]

Historical Information

In 1907, Ernest Edward Tyzzer provided a description of *Cryptosporidium* based on histologic sections of mouse intestine, in which the parasites were observed attached to the epithelial cells.[18] The pathogenic characteristics of *Cryptosporidium* were not recognized until much later, when D. Slavin, in 1955, established that this protozoan caused diarrhea in turkeys.[19] In 1976, F.A. Nime and coworkers described human diarrheal disease due to *Cryptosporidium*, and J.L. Meisel and colleagues, in 1976, were the first to report it in immunocompromised human hosts.[20, 21] Currently various species of *Cryptosporidium* are recognized as important causes 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 5 years of age.[24]

Life Cycle

Infection begins when the host ingests thick-walled sporulated oocysts (Fig. 10.1), each of

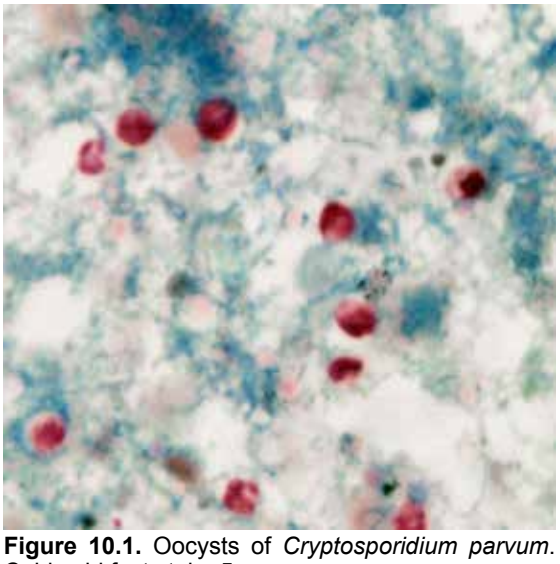


Figure 10.1. Oocysts of *Cryptosporidium parvum*. Cold acid fast stain. 5 μm.

which contains four banana-shaped sporozoites.[25] A minimum of 30 oocysts is necessary to initiate 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 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 inhibitor 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 (\ rɒptrīs\) 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, a purified microneme-specific mucin-like 900 kDa glycoprotein can prevent invading parasites from attaching to their target cells when employed in competitive inhibition studies.[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

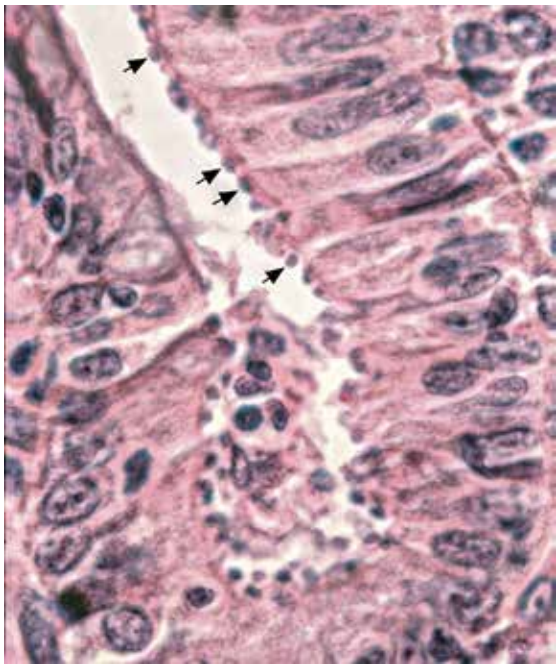


Figure 10.2. Histologic section of small intestine of patient suffering from HIV/AIDS, infected with *C. parvum* (arrows). Courtesy J. Lefkowitch.

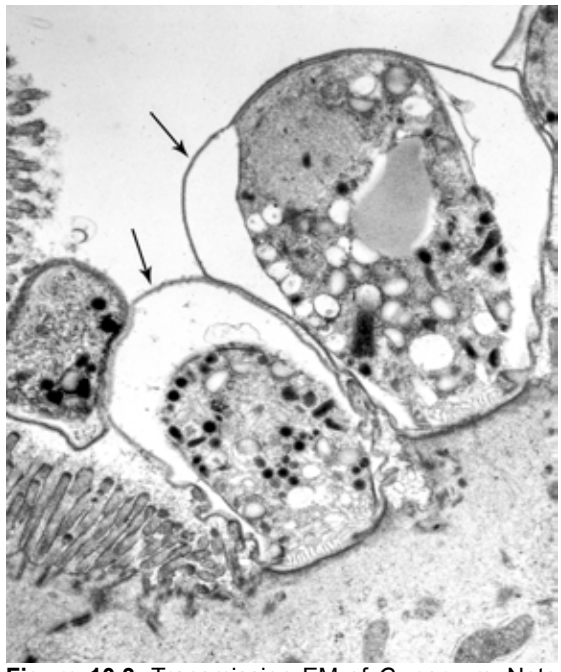
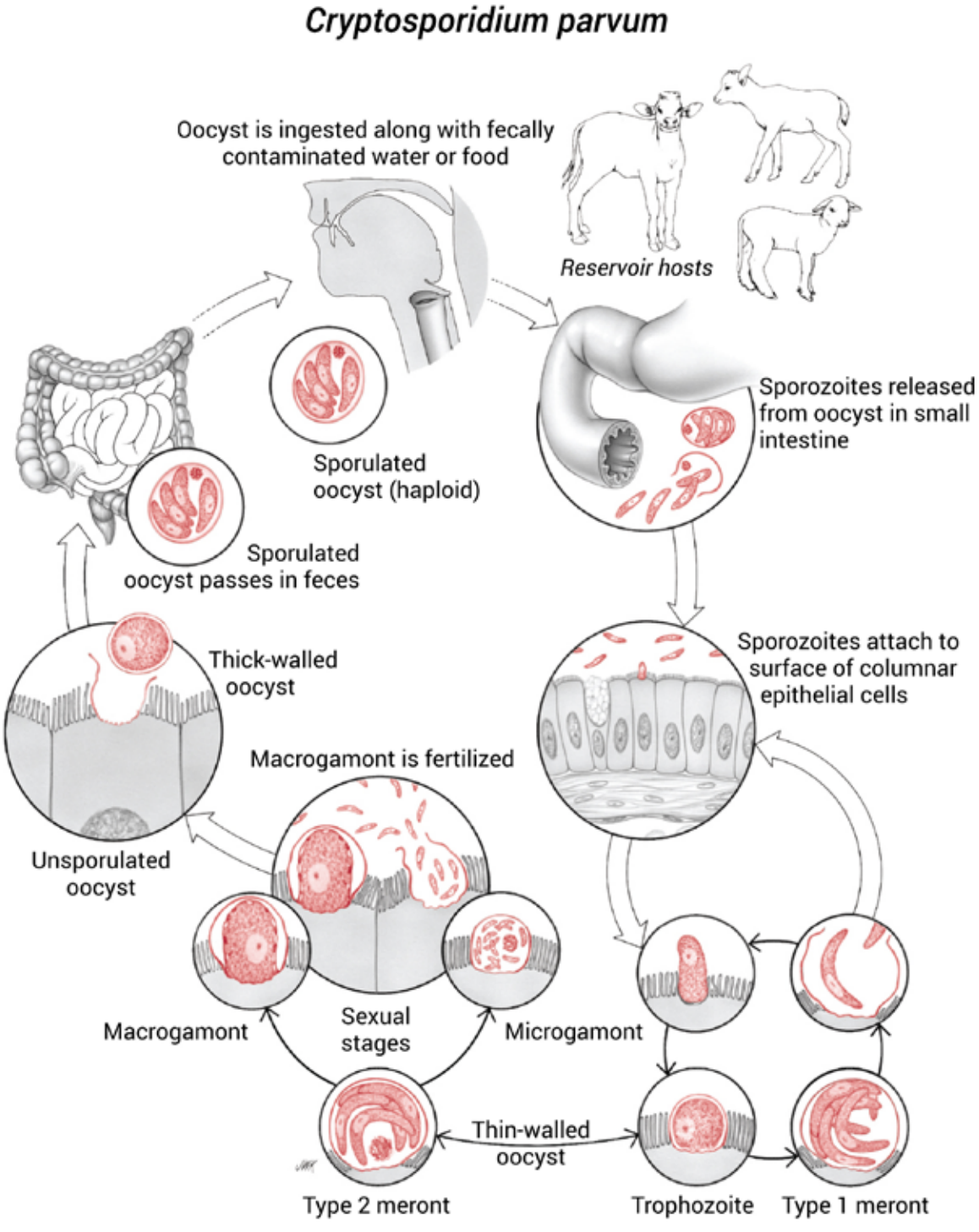


Figure 10.3. Transmission EM of *C. parvum.* Note microvillus-derived membranes encasing parasites (arrows). Courtesy J. Lefkowitch.


Cryptosporidium parvum
Oocyst is ingested along with fecally contaminated water or food
Reservoir hosts
Sporulated oocyst (haploid)
Sporozoites released from oocyst in small intestine
Sporulated oocyst passes in feces
Thick-walled oocyst
Sporozoites attach to surface of columnar epithelial cells
Macrogamont is fertilized
Unsporulated oocyst
Macrogamont
Sexual stages
Microgamont
Thin-walled oocyst
Type 2 meront
Trophozoite
Type 1 meront

to create a unique intracellular environment (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 there by means of 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 was able to complete its development 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. Following their release, microgamonts fuse with macrogamonts, forming thick-walled zygotes termed oocysts. This stage sporulates within the large intestine, and four haploid infectious sporozoites are produced. 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.

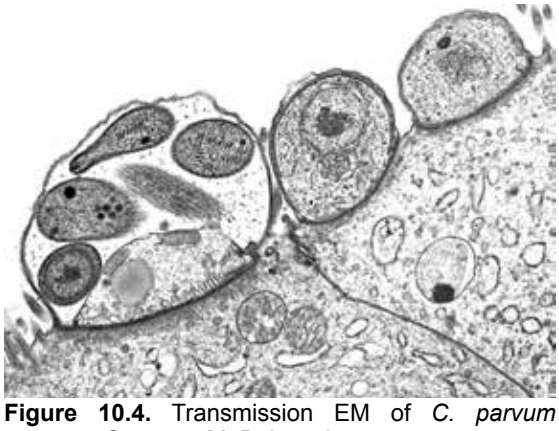


Figure 10.4. Transmission EM of *C. parvum* meronts. Courtesy M. Belosevic.

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] The altered microvillus-derived membrane complex that surrounds the parasites while they are attached to epithelial cells 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 surface of the host cell. 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 kind of platform on top of which the organism then elaborates its complex of membranes. Much more needs to be learned about the mechanism(s) of nutrient acquisition by *C. parvum* before rational drug design, aimed at interference with this process, can evolve.

Although not fully understood, the secretory diarrhea and pathogenesis of this organism are being deciphered now that the genome is fully sequenced.[16, 17] More than 25 pos-

sible factors responsible for virulence have now been isolated, and their specific roles in causing damage and diarrhea are being examined.[23] Certain virulence factors appear to be involved in: 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 of host cells.[44] In addition to microbial virulence factors, pro-inflammatory cytokines such as interleukin-8 are possibly involved.[45]

Protection against the primary infection develops in individuals whose immune systems are not compromised. At least two classes of antibodies, IgA and IgG, and several cellular-based immune mechanisms are thought to play important roles in the elimination of the parasite from the gut tract, although 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] It has also been observed that patients living in areas endemic for *C. parvum* have milder symptoms with repeated infections.[48] Studies carried out in experimental infections employing various strains of inbred mice have shown that interleukin-12, interferon-γ, and perhaps β-defensins, peptides chemically related to magainins, act in conjunction to protect against a 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 infection.[54] Patients suffering from AIDS may develop an antibody response that is measurable in both serum and intestinal secretions, but this does not allow them 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 is usually initiated through ingestion of contaminated water, directly from an infected person or animal, in tainted food, or rarely through aerosol.[59] In immunocompetent individuals disease can vary from asymptomatic infection to mild or profuse watery diarrhea. Upper abdominal cramps, anorexia, nausea, weight loss, and vomiting are common features of the acute stage of the infection.[60] Severity of disease does not appear to correlate with the intensity of exposure.[26] In those who have already experienced clinical disease and recovered, a second infecting dose of oocysts may be asymptomatic, or they may have only a mild, transient diarrhea. Cryptosporidiosis in an immunocompetent host is self-limited, lasting for up to 2 weeks, but may persist for longer periods in some individuals. In others, diarrhea may be severe with several liters per day of diarrhea, and even persistent diarrhea with impacts on 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 5 years of age.[24] Those undergoing cancer chemotherapies suffer worse yet, with protracted, life-threatening diarrhea accompanied by significant weight loss.[63]

Cryptosporidiosis in patients suffering from 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 mal-

nutrition 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 sugar solution, then stained by acid-fast methods.[67] PCR testing is highly sensitive and can identify specific genotypes, which is 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 both feces and tissue specimens.[70] The introduction of multiplex NAAT testing has helped to increase the number of cases of infectious diarrhea now recognized to be due to *Cryptosporidium* spp.[24] This approach to the diagnosis of infectious diarrhea is now a highly sensitive and commercially available diagnostic option for the diagnosis of cryptosporidiosis.[71]

Treatment (see Clinical Appendix)

Treatment of cryptosporidiosis is based on 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 initiation of HAART to reconstitute their immune systems. All patients should receive supportive care with oral rehydration when possible and intravenous therapy if required. Several clinical trials with rifamycins, azalides and paromomycin have demonstrated no clear benefit illustrating the difficulty of treating acute or established disease.[75]

Prevention and Control

Good hygiene is always an important approach to decreasing one's risk of exposure. Without knowledge as to the source of a given outbreak, control and prevention of infection due to *C. parvum* is not possible. In the case of waterborne epidemics, management of watersheds is the long-term solution in situations where the water supply is not filtered.[12, 76] Filtering drinking water is usually effective, but deterioration of filtration equipment and/or lack of proper maintenance can erode any progress made in controlling waterborne infections. Boiling is another option for purification of contaminated drinking water. Chlorination of water supplies is ineffective against the oocyst, but ozonation kills this stage.[77, 78] In agricultural settings, creation of vegetative barriers to curtail the spread of oocysts is effective.[79] Surveillance is key to keeping public water supplies free of pathogens with environmentally resistant stages (e.g., *Giardia lamblia, Entamoeba histolytica, C. parvum*). In this regard, PCR-based testing now allows for the possibility of continuous monitoring of water supplies for *C. parvum*.[80] Urban and suburban pet stores and petting zoos for children are other sources of infection that until recently have received little attention.[81]

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11. *Toxoplasma gondii*

(Nicolle and Manceaux 1908)

Pronunciation: \täk-sə-ˈplaz-mə\\ gon'dē-ī\

Introduction

Toxoplasma gondii (\TOK-so-plaz-ma\\gon-DEE-eye\) is an obligate intracellular parasite that has 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. *T. gondii* infects most species of 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, even when one takes into account all the viruses and bacteria that infect this large group of vertebrates. 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 life of the host. 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 the ingestion of 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 and can occasionally lead to devastating pathological consequences for the developing fetus.

Historical Information

In 1908, Charles Nicolle and Louis Manceaux described the organism which they isolated from the gondi (*Ctenodactylus gondii*), a gerbil-like desert inhabiting mammal.[12] In the same year, Alfonso Splendore, working in Brazil, described the identical parasite, which he identified in the tissues of rabbits.[13] They published their results at the same time, 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 was unable to isolate the organism from the brains of its victims. In 1939, Abner Wolf and colleagues confirmed Janku's clinical description, and went on to experimentally transfer the infection from infected brain tissue to mice and rabbits.[15] In 1941, H. Pinkerton and 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 fed to them by their unwitting owners 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 main 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 paths of development, 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 infectious 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 they 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 for 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, full 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 sexual development of *T.*

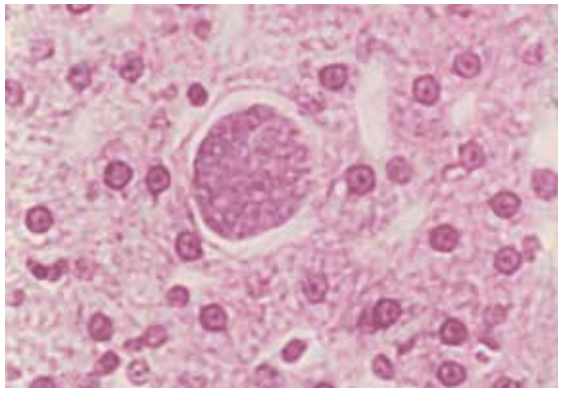

Figure 11.2. Pseudocyst of *T. gondii* in liver biopsy.

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

gondii in the feline intestinal tract. [23]

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

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 exposure of the oocyst to digestive enzymes in the small intestine. The freed parasites then penetrate the intestinal wall and are taken up by macrophages. *T. gondii* now can follow the extra-intestinal pathway.

Once inside a cell (Fig. 11.3), the organisms are referred to as tachyzoites. This stage resides in its own membrane-bound parasitophorous vacuole.[24] Infected cells are not able to destroy tachyzoites, due to the fact that *T. gondii* inhibits the process of fusion 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 site of release (e.g., glial cells, astrocytes, hepatocytes, neutrophils, cardiac muscle). *T. gondii* undergoes another round of replication until protective immune responses are elicited. As the 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.

Apparently, protective immunity does not result in the elimination of the parasite. Rather, in response to host defense mechanisms, tachyzoites are forced to differentiate into a second asexual stage known as the bradyzoite. This form divides both by endodyogeny and endopolygeny, then organizes into a tissue cyst.[27] Interferon-γ-dependent, NO-mediated

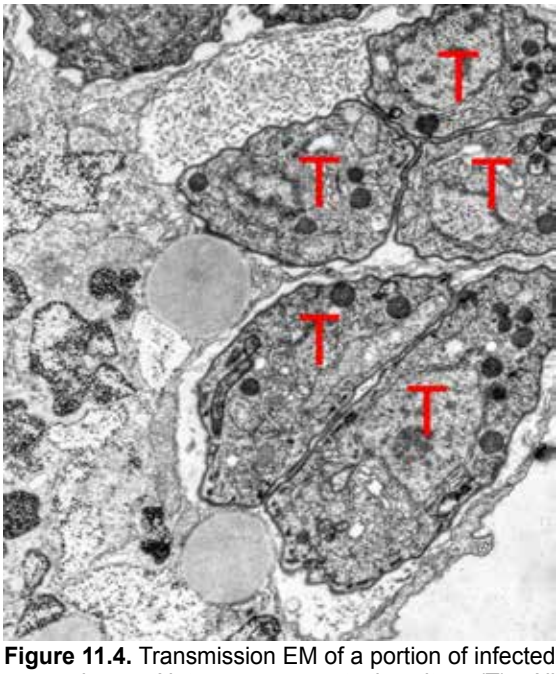

Figure 11.4. Transmission EM of a portion of infected macrophage. Note numerous tachyzoites (T). All parasites are alive; thus, the fusion of lysosomes with the parasitophorous vacuole is inhibited. Courtesy T. Jones.

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

effector mechanisms maintain this state of latency by eliminating any parasites emerging from the cysts.[28] Bradyzoites lie dormant in the tissues for 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 as well as paratenic hosts can be infected by ingesting tissue cysts contained 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 cooking of beef to kill *T. gondii* is the final cooked temperature of a medium rare steak.

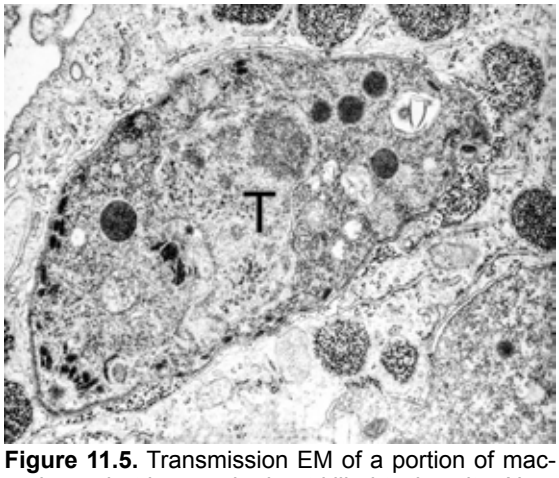

Figure 11.5. Transmission EM of a portion of macrophage that ingested a heat-killed tachyzoite. Note fusion of lysosomes with the parasitophorous vacuole (see top right fusion event. T = tachyzoite, Courtesy T. Jones.

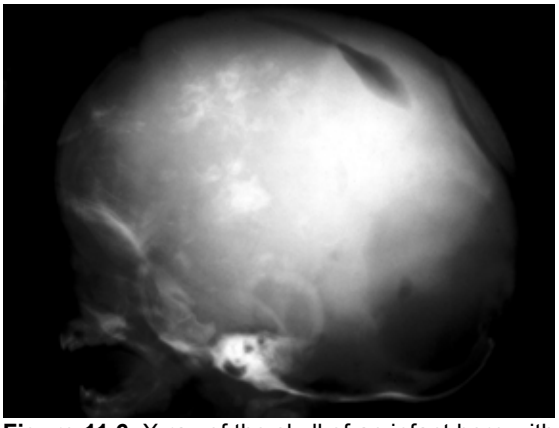

Figure 11.6. X-ray of the skull of an infant born with congenital toxoplasmosis. Infection was acquired most likely during the first or second trimester. Note calcifications.

Congenital transmission may occur from an infected mother to 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 to be necessary in preventing 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. The process is nevertheless complex, and involves the coordinated, sequential deployment of a set of specialized subcellular organelles: micronemes, rhoptries (lysosome-like granules), dense granules and the glideosome.[34, 35] As the result of the biological activities unleashed upon the host cell by these organelles, the tachyzoite is able to assume its intracellular life without hindrance from host defense mechanisms related to phagocytosis. *T. gondii* employs an entry scheme referred to as a 'kiss and spit' approach, whereby it sprays virulence factors onto potential host cells, as well as into already infected host cells.[36] Rhoptries, located at the apical end of

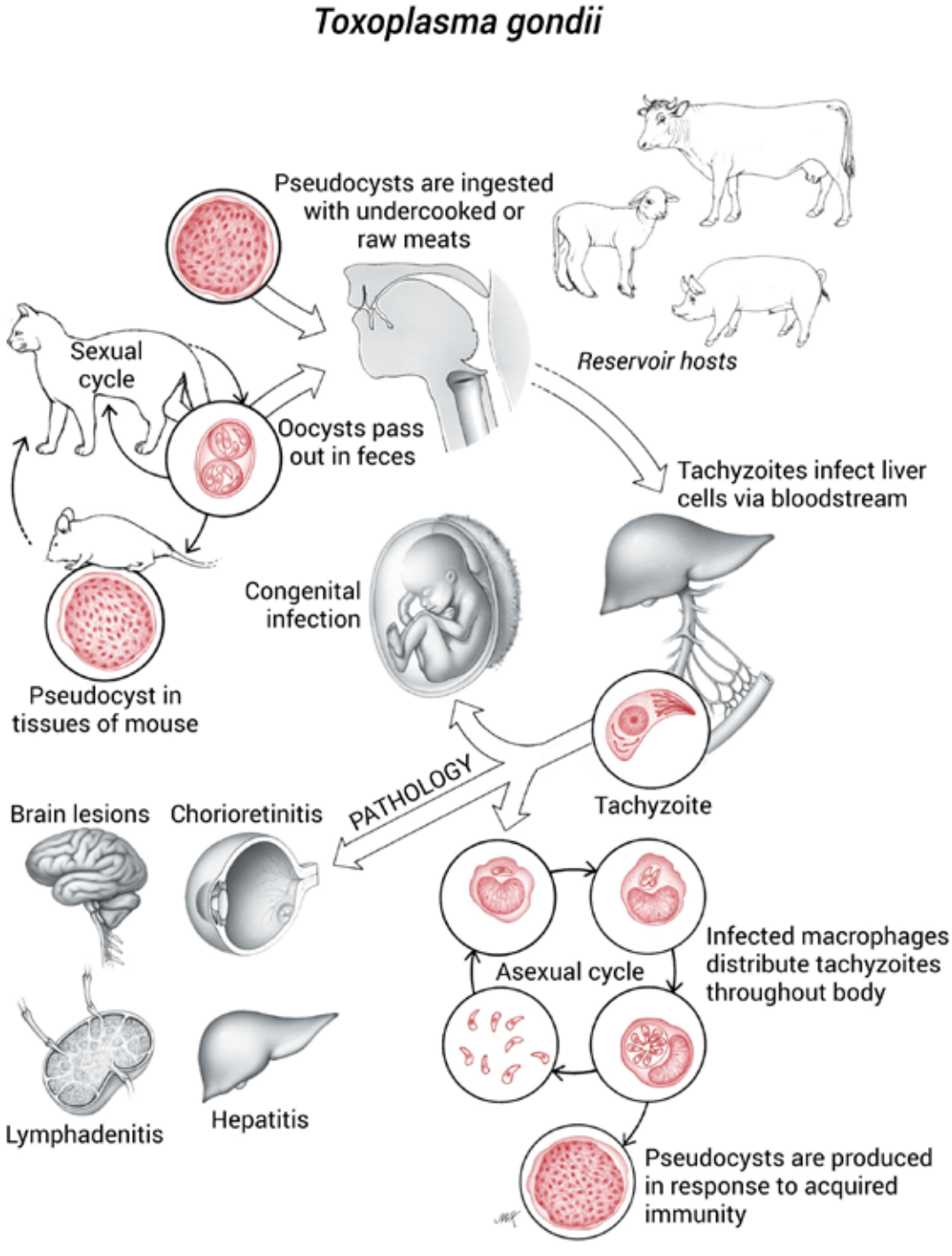
Toxoplasma gondii
Pseudocysts are ingested with undercooked or raw meats
Reservoir hosts
Sexual cycle
Oocysts pass out in feces
Tachyzoites infect liver cells via bloodstream
Congenital infection
Pseudocyst in tissues of mouse
Tachyzoite
PATHOLOGY
Brain lesions
Chorioretinitis
Infected macrophages distribute tachyzoites throughout body
Asexual cycle
Lymphadenitis
Hepatitis
Pseudocysts are produced in response to acquired immunity

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

the tachyzoite, and micronemes both secrete adhesin-like molecules.[35, 37-40] Parasite-specific secreted serine and cysteine proteases are required for the engineering of the parasitophorous vacuole in which the tachyzoite lives and reproduces.[37, 41, 42] These secreted serine and cysteine proteases are released and enable the parasite to deform the cell membrane of the target cell and re-model the inner membrane of the vacuole. 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 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 infiltration of inflammatory cells. Eventually, granulation tissue forms, and invades the vitreous humor. Calcification of brain tissue (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

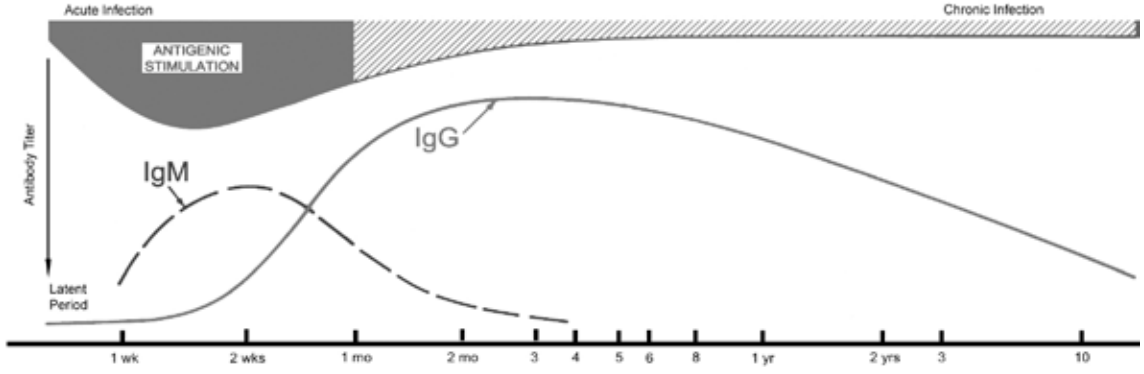

Figure 11.7. Relationship between antigenic stimulation, antibody production, and stages of infection (latent period, acute, and chronic infection). Redrawn after J. Remington.

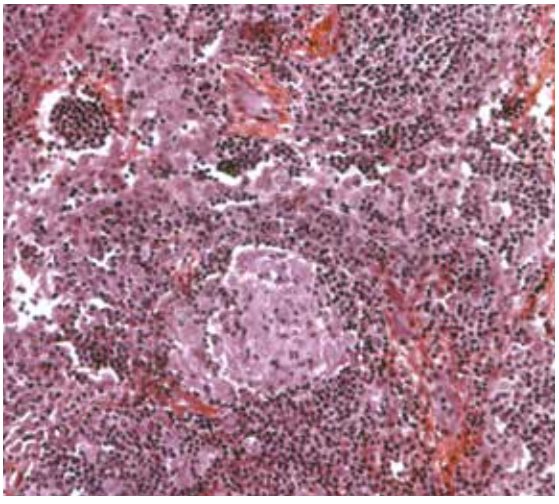

Figure 11.8. Histologic section of lymph node positive for macrophages infected with *T. gondii*. These cells are referred to as Piringa-Kuchenka cells.

or second trimesters have been documented but are less common for those whose infection 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 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 due largely to the fact that HIV down regulates interleukin-12 production and reduces the number of parasite-specific $CD4^+$ and $CD8^+$ T cells.[54-56] This reduces dramatically 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, the incidence of 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 stillbirth (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 the majority of congenital toxoplasmosis with most affected children asymptomatic. They may show the less severe pathological consequences of infection several months to years later. The classic triad of chorioretinitis, hydrocephalus, and intracranial calcifications 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 has gone unnoted and untreated.[64] Of the infants that do 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 that severe impairment to the CNS will occur.[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 reactivation of latent *T. gondii* acquired previously. HIV-infected patients at significant risk are those with $CD4^+$ T cell counts <100 cells μl who have 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 an interstitial pneumonitis.[51, 52] The GI tract, liver, and heart may also be involved. Cutaneous toxoplasmosis presents 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 a 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 by using PCR tests.[76-79] PCR tests are especially useful in identifying organisms in ocular, amniotic, or CSF fluids.[80] Identification of tachyzoites in tissue sections without the aid of antibody-based staining methods is very challenging, even for a seasoned histopathologist. Indirect evidence of infection includes the application of 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. A wide variety of laboratory-based methods take advantage of these host responses to insure an accurate diagnosis of both 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 thereafter. 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 is also diagnostic.

In certain regions of the world, women at increased risk are recommended to undergo screening during pregnancy.[82] Sero-conversion during pregnancy can then be documented early on in infection and closely mon-

itored. 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 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 administration of 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/μl.[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 infection of the fetus. In most cases pyrimethamine and sulfadiazine are the drugs of choice.[84] For a number of reasons, alternative, less studied regimens are often necessary. If pyrimethamine is not available 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. The combination of pyrimethamine and sulfadiazine is teratogenic in animals and there is concern regarding its use in pregnancy, particularly during early trimesters. As a consequence, women who become acutely infected during pregnancy are often treated with the macrolide antibiotic spiramycin. The ability of this agent to concentrate in the placenta is thought to help prevent transmission to the fetus.[86, 87] Some physicians will treat women who acquire acute infection during later trimesters with pyrimethamine and sulfadiazine, but in some settings women will choose to terminate their pregnancies, as it is not clear that initiation of treatment after the development of intracranial calcifications has any benefit.[88]

Prevention and Control

Eating only well-cooked meats and avoiding the inadvertent 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 the prevalence of infection is over 85% among those over the age of 50, the most common source of infection is raw meat served as "steak" tartar, most of which is actually lamb or horsemeat. Many other cultures also have numerous recipes calling for undercooked or raw meat as a main ingredient. A number of Alaskan native peoples still eat some of their meat raw, and are therefore at the mercy of the pathogens lurking inside each carcass.[89] In Ethiopia, the practice of eating raw meat with melted butter, *kitfo*, is a popular but risky dietary choice.[90] Prevention of infection from oocysts requires care when handling cat feces, especially when cleaning litter boxes.[91] Pregnant women diminish their risk of infection by avoiding these activities. Rarely, toxoplasmosis is acquired as the result of inhaling dust, or drinking water contaminated with oocysts.[92] Vaccines potentially offer another set of strategies for controlling this infection, but carefully defined susceptible at-risk

populations, such as sero-negative women of child-bearing age, would need to be clearly articulated prior to committing the necessary resources to vaccine development.[93]

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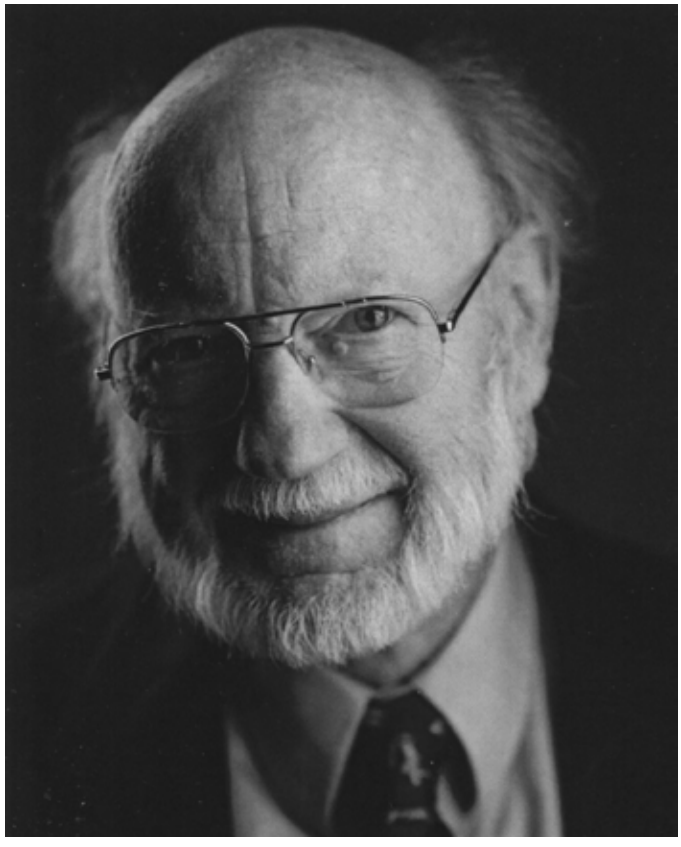


William Cecil Campbell, Ph.D. (born 1930)

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-ˈmē-bə\\ hisʹtəlitʹikə\

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 species of amoebae are free-living, many 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, but none other than *E. histolytica* are clearly pathogenic. There is some controversy regarding the role of *E. moshkovskii* in childhood diarrhea, particularly in Bangladesh.[2, 3]

E. histolytica is transmitted from person to person via the fecal-oral route, taking up residence in the wall of the large intestine.[4, 5] Protracted infection can progress from watery diarrhea to dysentery (bloody diarrhea) that may prove fatal if left untreated. In addition, *E. histolytica* can spread to extra-intestinal sites causing serious disease.[6] *E. histolytica* lives as a trophozoite in the tissues of the host and as a resistant cyst in the outside environment. Sanitation programs designed to limit exposure to food and water-borne diarrheal disease agents are effective in limiting infection with *E. histolytica*. Some animals (non-human primates and domestic dogs) can become infected with *E. histolytica*, but none serve as important reservoirs for human infection.

E. dispar and *E. moshkovskii* are morphologically identical amoebae that can be misidentified as *E. histolytica* during microscopic examination of fecal samples.[7] Commercially available stool antigen detection tests and NAATs are now widely available that are sensitive and specific, allowing for detection of *E. histolytica* and discrimination between *E. histolytica* and nonpathogenic amoebae.[8, 9] Much is now known about the basic biology and clinical aspects of infection with *E. histolytica,* and the genome has been sequenced.[10-12]

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.[13, 14] In 1893, Heinrich Quincke and Ernst Roos distinguished *E. histolytica* from *Entamoeba coli*, a non-pathogenic amoeba acquired by the fecal-oral route and often found in the stool of asymptomatic individuals.[15] In 1903, Fritz Schaudinn described the trophozoites and cysts of *E. histolytica*.[16] He died at the age of 35 of overwhelming amoebiasis, a tragic consequence of self-experimentation. In 1891, William Councilman and Henri Laf-

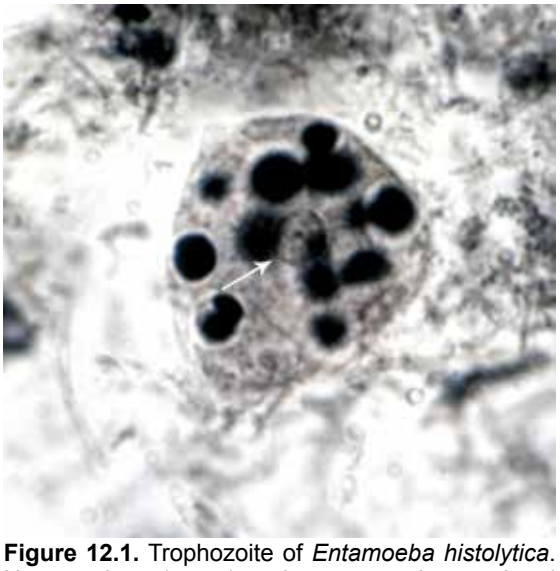


Figure 12.1. Trophozoite of *Entamoeba histolytica*. Note nucleus (arrow) and numerous ingested red cells. 35 μm.

leur described the main features of the intestinal pathogenesis caused by *E. histolytica*. In 1925, William Boeck was the first to culture *E. histolytica*, while Clifford Dobell, in 1928, fully elucidated its life cycle.[17-19]

Life Cycle

The trophozoite (Fig. 12.1) is a facultative anaerobe metabolizing glucose as its main source of energy.[20] 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.[21-23] *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.[24, 25] Evidence suggests that the mitosome no longer contains any DNA and likely lost its genome through reductive evolution.[24] Discovery of a calreticulin-like molecule of 51 kDa suggested the presence of an endoplasmic reticulum (ER) and further work has now verified the presence of a continuous ER in *E. histolytica*.[26, 27]

The cyst (Fig. 12.2) is smaller than the trophozoite (10–15 μm in diameter), and at full maturity contains four typically round *E. histolytica* nuclei. Each nucleus ultimately will give rise to an individual trophozoite. Immature cysts may contain 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 all that is necessary to initiate infection, making this organism one of the most efficient pathogenic protozoa known to infect humans.[28] Each cyst undergoes excystation in the small intestine. Excystation is complex, involving actin cytoskeletal reorganization.[29, 30] The cyst must receive certain specific environmental cues from the host, including sequential exposure to an acidic and a basic pH environment, in order for the single quadrinucleate cell within the cyst to undergo cytokinesis, exit through the cyst wall, and enter the small 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.[6]

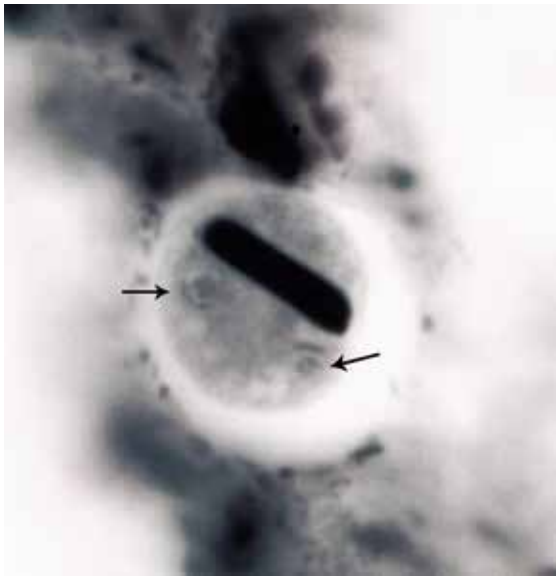


Figure 12.2. Cyst of *E. histolytica*. Two nuclei (arrows) and a smooth-ended chromatoidal bar can be seen. 15 μm.

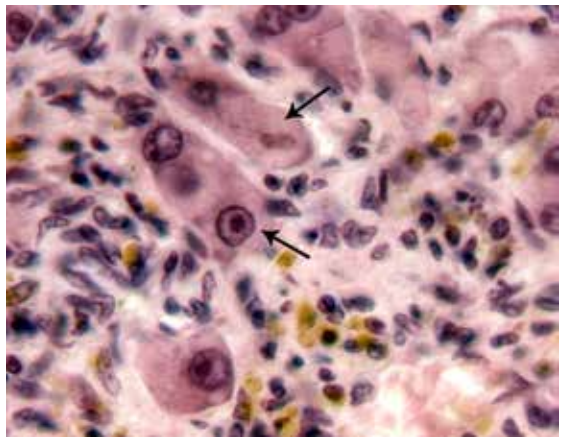
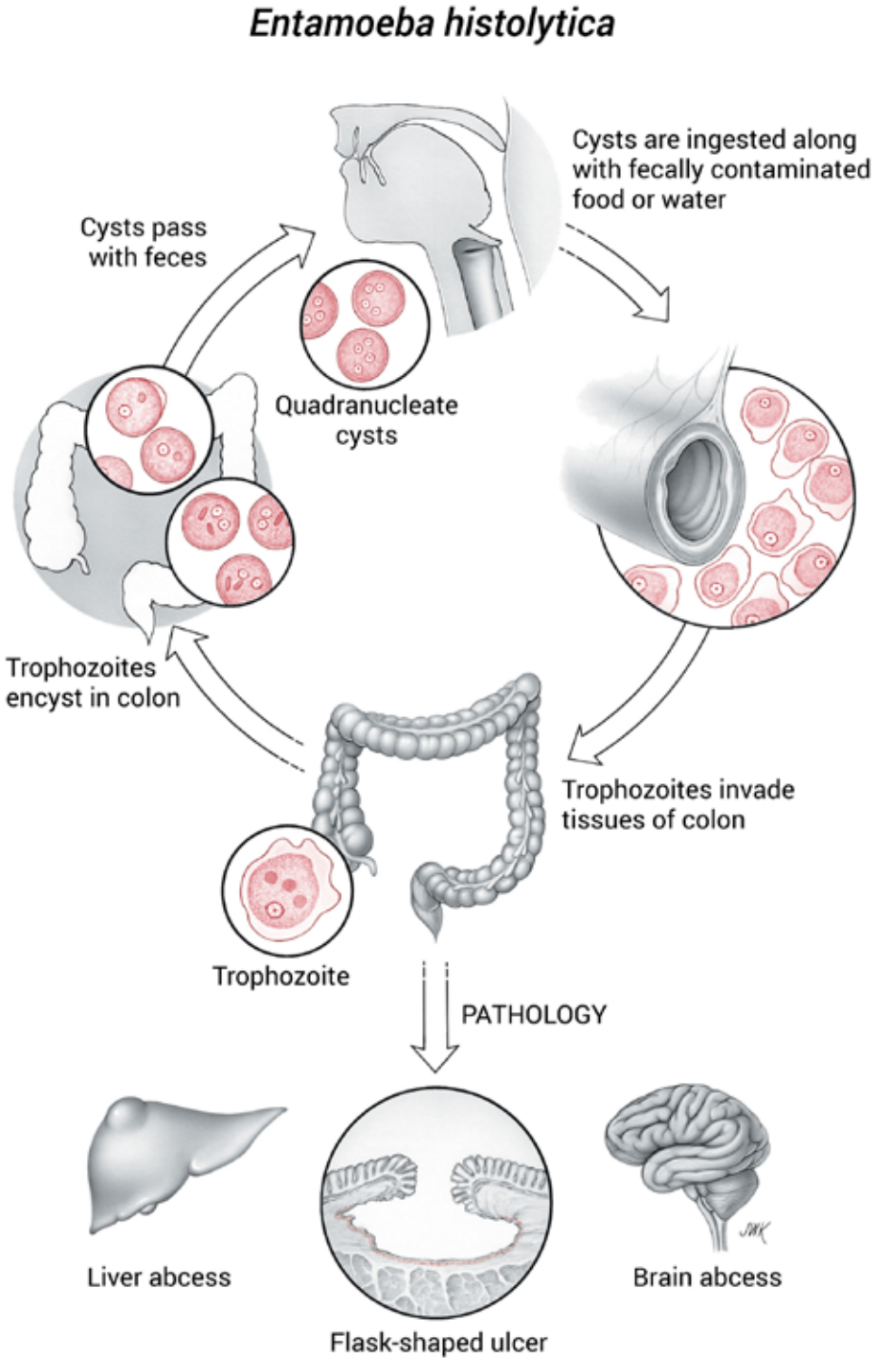


Figure 12.3. Trophozoites of *E. histolytica* in liver abscess (arrows). Note ingested host cells inside parasites.


Entamoeba histolytica
Cysts are ingested along with fecally contaminated food or water
Cysts pass with feces
Quadranucleate cysts
Trophozoites encyst in colon
Trophozoites invade tissues of colon
Trophozoite
PATHOLOGY
Liver abcess
Flask-shaped ulcer
Brain abcess

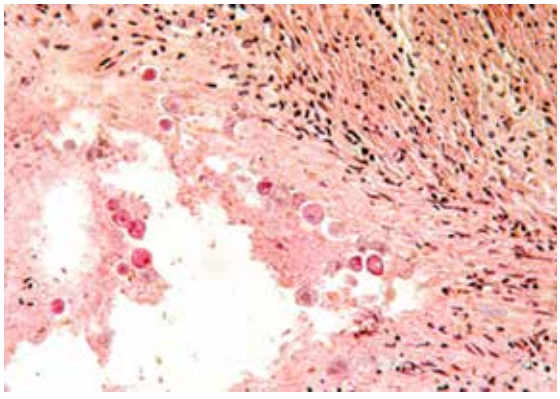


Figure 12.4. Low-magnification histologic section of amoebic ulcer in small intestine. Organisms can be seen at living margin of ulcer.

In the large intestine the trophozoite penetrates the perimucosal space and attaches to epithelial cells using lectin-galactose interactions.[31] This event is cytotoxic.[32] The amoeba engulfs and kills only living cells (Fig. 12.3).[31] Trophozoites divide by binary fission, occupying increasingly larger areas of tissue as they do so.[19] This activity eventually causes flask-shaped ulcers to develop (Fig. 12.4). Hematogenous or lymphatic spread is then 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 that is likely triggered by means of the Gal/Gal-NAc-specific lectin.[29, 30] Amoebic proteasome activity may also be necessary for the process, since treating cultures with lactacystin caused marked inhibition of cyst formation.[33, 34] 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.[35] During infection in the gastrointestinal (GI) tract, cysts may be continuously produced 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.[11, 12, 36-40] Amoebae must attach to host tissues as a necessary prerequisite for parasite-mediated cytotoxicity. Attachment is dependent upon interactions between epithelial cell membrane-bound N-acetyl-glucosamine and N-acetyl-galactosamine and at least two surface lectin proteins.

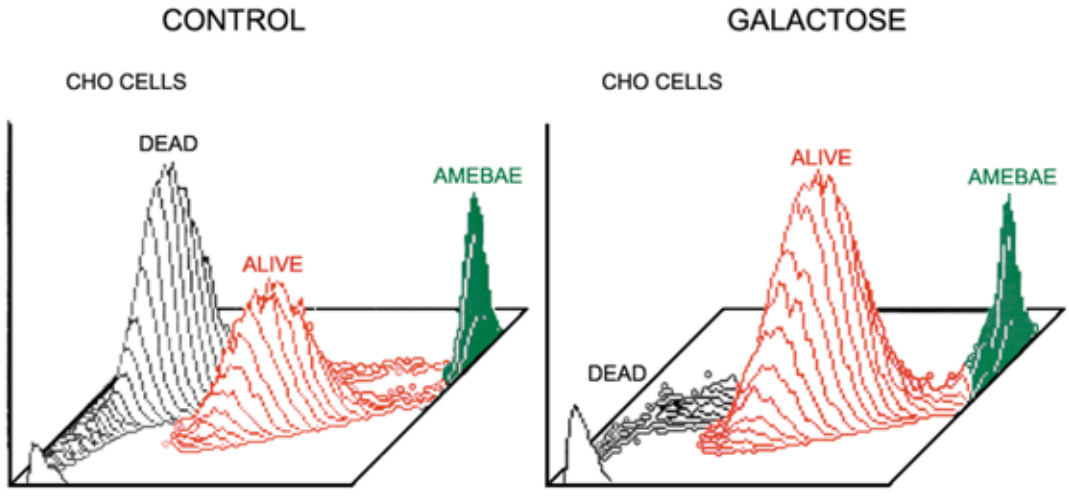


Figure 12.5. *In vitro* experiment showing that galactose-containing surface proteins are important for parasite cytotoxicity. Free galactose prevents attachment of amoebae to their target cells. CHO cells=Chinese hamster ovary cells. Redrawn after J. I. Ravdin.

The genes for both of the parasite lectins have been cloned and their cDNAs sequenced. One lectin is a 260 kDa protein, while the other is 220 kDa.[41] 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.[42, 43] These surface lectins apparently also facilitate the parasite's evasion of the complement membrane attack complex.[44]

In vitro, *E. histolytica* can be inhibited from attaching to its target cells simply by adding free galactose to the medium (Fig. 12.5).[45] In this situation, cells and trophozoites coexist. Attachment leads to cell death, which, at least *in vitro*, is calcium-dependent.[46] Several possible mechanisms for the actual killing of host cells have been proposed, all of which involve enzymes such as serine proteases.[47] A recent finding has shown that trophozoites can "nibble" off a portion of their target cells just before actually killing them; a process termed trogocytosis.[48] Apparently, *E. histolytica* needs to sample its meal before fully committing to ingesting it. This pathogen may turn out to be a fussier eater than previously thought. Although inflammation and the release of cytokines may play a role in the tissue destruction associated with acute infection, there is a significant amount of tissue destruction present in chronic ulcerations that is out of proportion to the degree of inflammation seen at this stage of infection.[49]

The trophozoite's surface membrane contains phospholipase A, neuraminidase, and a metallocollagenase.[50] In addition, it secretes a minimum of four cysteine proteases.[51] 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.[52, 53] 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.[54] 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 lymphotactic interleukin-8 in the surrounding colonic epithelial cells, while simultaneously inhibiting the upregulation of other cytokines known to play a role in inflammation.[55, 56]

Protective immune mechanisms are short-lived and depend on the development of secretory IgA antibodies directed against parasite surface proteins involved in adherence to target cells such as Gal/GalNAc lectin, a novel multifunctional virulence factor.[57-61] In addition, cell-mediated killing of parasites can occur by induction of NO by the 220 kDa lectin, which up-regulates interferon-γ.[62, 63] 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.[64] In experimental infections, polyclonal and monoclonal antibodies have been shown to be effective in protecting the host when they are directed against carbohydrate-binding lectins of the parasite, emphasizing the central role these parasite proteins play in the pathogenesis of disease.[65, 66]

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, as well as the severity and type of manifestation.

Those who are symptomatic may experience

a wide range of clinical disease.[38, 67] The most common manifestation is diarrhea, lasting more than a few days, and in some cases for weeks, months or years if not treated appropriately.[38, 67] Although a large number of patients do not report blood or excess mucous in their stools, almost all stools are heme-positive if tested.[68] 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, a large percentage of patients will report fever at clinical presentation.[68]

Generalized abdominal tenderness, with particular accentuation in the iliac fossae 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 the chronic condition, amoeboma (large granuloma consisting of eosinophils, amoebae, and necrotic colonic tissue) are possible, presenting as palpable masses. Amoeboma are often misdiagnosed on barium enema as malignancies. If disease progresses, the colon may become atonic and may 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.[38]

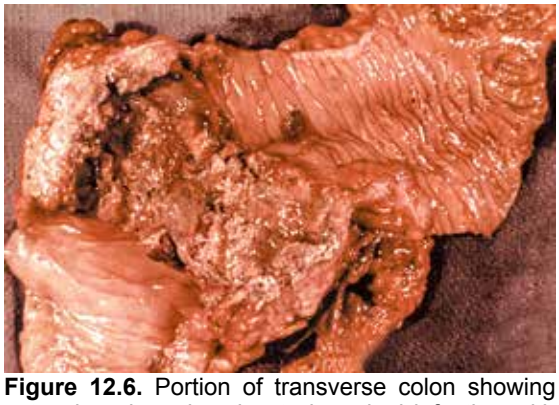


Figure 12.6. Portion of transverse colon showing extensive ulceration due to intestinal infection with *E. histolytica*.

The perforated, inflamed bowel may adhere to the abdominal wall, and the perforation may extend to the skin, causing cutaneous amoebiasis, which can progress rapidly.[69] This situation may also occur in the perianal area as the result of invasion of the skin 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.[67, 70] 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 abscess 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 to the shoulder region. Examination at that time may reveal only a slightly enlarged, tender liver, or it may reveal a mass. In some cases pressure exerted between the ribs, the so called 'intercostal sign', will detect tenderness.[6] Most patients with hepatic amoebiasis have involvement of the right hepatic lobe, but the left lobe of the liver can also be infected; the enlargement and tenderness can be central or even left-sided.

The lungs are the next most common extra-

intestinal sites of infection.[71, 72] 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. 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 on in the infection.[73]

It is now recognized that many populations infected with HIV-1 have higher exposures and asymptomatic colonization rates for *E. histolytica.* It is not clear whether there is a significantly higher rate of progression to invasive disease in these patients compared to those without HIV infection but the higher incidence in this population increases the number of infected individuals potentially progressing to invasive disease.[74-77]

Diagnosis (see Clinical Appendix)

The site, as well as duration of infection, dictates the most appropriate diagnostic test. Stool testing for heme is positive in almost all cases of intestinal amoebiasis if performed properly, while stool tests for leukocytes tend to be negative due to the ability of the amoeba to destroy leukocytes despite the invasive nature of this disease. Definitive diagnosis depends upon: detection of antigens in stool, NAAT on stool or tissue samples, or microscopy coupled with species identification by

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 μm.

one of the first two testing modalities.[8, 78] 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 non-pathogenic *doppelgängers*.[8] Molecular testing with NAAT is reported to have a sensitivity 100x that of stool antigen testing, and is now available and in routine use in many centers as part of a gastrointestinal (GI) diagnostic panel.[74, 79]

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*.

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.[80-83] If red blood cells are seen in the cytoplasm, particularly more than 2 erythrocytes per trophozoite, then *E. histolytica* is more likely (Fig. 12.7).[83]

The Charcot-Leyden crystals (Fig. 12.8) in stool are frequently present when patients are suffering from disease caused by *E. histolytica*, but they are also seen with heavy infection caused by *Trichuris trichiura* and *Strongyloides stercoralis*, and therefore are not pathognomonic for amoebiasis. PCR can also be useful for diagnosis of liver disease when used on aspirates derived from the abscess.[84]

Since infection with *E. histolytica* invariably leads to long-lasting antibody production, antibody-based tests are sometimes difficult to interpret, especially when done during chronic infection.[85] Serological testing will become positive, even in intestinal amoebiasis infection, 5–7 days after onset of symptoms and usually remain positive for years, consequently limiting their utility in endemic areas where up to 35% of the population may have seropositivity due to prior exposure.[86] The indirect hemagglutination assay (IHA) and indirect fluorescent antibody (IFA) test are used together to rule in the possibility of extra-intestinal disease, but are not definitive proof of infection. Intestinal amoebiasis must always be considered in any patient with protracted diarrhea and in all patients with dysentery. The diagnosis must also be considered in patients presenting with intraluminal colonic masses, because of the development of amoebomas that resemble carcinoma of the colon. 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 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 the setting of extra-intestinal amoebiasis. Serology is usually positive in cases of extra-intestinal amoebiasis, but may be negative during the first 7 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. On ultrasonography, an amoebic liver abscess usually appears as a round hypodense area that is contiguous to the liver capsule, usually without significant wall echoes.[87] 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.[88]

If the diagnosis is still in doubt after serological testing and imaging, the cyst can be aspirated. The obtained fluid from a hepatic amoebic abscess is often a brown fluid containing necrotic hepatocytes that has been likened to anchovy paste. In some cases, the fluid may be clear or yellow in color. Amoebic

forms are rarely present and antigen testing or NAAT is helpful in confirming the diagnosis. Abscesses may be secondarily infected with bacteria, so sending the fluid for bacterial culture is recommended. 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.[89-91]

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 that reaches adequate levels at the sites of infection. Metronidazole is the drug of choice for the intestinal and extra-intestinal infection.[92] It can be given in equivalent doses orally or intravenously. 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. Since these drugs do not target the cyst stage, a second intraluminal agent to target the intraluminal cysts is also recommended for all symptomatic patients. Treatment of asymptomatic carriers of *E. histolytica* to prevent disease or transmission is controversial.[93] A cysticidal intraluminal agent alone may be adequate for asymptomatic cyst passers and those with nondysenteric amoebic colitis. The intraluminal agent paromomycin is widely used in the United States for this indication, while other agents, such as diloxanide furoate and iodoquinol (diiodohydroxyquinoline), are both effective at killing cysts and should be considered.[94] Additional antibiotics are appropriate in patients in whom a secondary bacterial infection is suspected.

No naturally occurring metronidazole-resistant strains of *E. histolytica* have been reported to date, but they can be easily induced under laboratory conditions.[95, 96] It is probably only a matter of time before they appear in human populations. Liver abscesses resolve slowly, despite treatment with the recommended high doses of metronidazole. Aspiration of an amoebic liver abscess may serve not only to confirm the diagnosis, but also as a therapeutic intervention. There are established indications for therapeutic drainage of an amoebic liver abscess and for surgery, in cases of severe colitis. 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.[67, 97, 98]

There are few reports of patients surviving amoebic abscess of the brain, since, unfortunately, they are typically diagnosed too late.[73] 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.[99]

Prevention and Control

Good public health practice, 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 whose occupations would place the general public at risk. 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. All infected individuals should receive treatment.

Vaccine targets against both the intestinal and extra-intestinal infection have been identified. Successful development of vaccines based on these findings will require extensive non-clinical and clinical studies before a vaccine is considered for the highest risk groups including children in endemic areas and other high-risk groups such as travelers.[100]

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13. *Balantidium coli*

(Malmsten 1857)

Pronunciation: \bal-ən-ˈtid-ē-əm\\kō-ˌlī\

Introduction

Balantidium coli (\BAL-an-TID-ee-um\\ KOHL-eye\) is the only ciliated protozoan that routinely infects humans. Balantidiasis occurs throughout the world, but the prevalence of human infection is not known. It is endemic in Japan, New Guinea, Micronesia, Seychelles Islands, Thailand, South Africa, Central and South America, and Europe.[1-6] There is controversy about whether the pig-associated organism, *Balantidium suis* (\ SW-ee\, \swʸē\), and the human-associated organism, *B. coli,* are the same species.[7]

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 both domestic and wild mammals (non-human primates, guinea pigs, horses, cattle, pigs, wild boars, and rats).[1] When patients suffering from HIV encounter *B. coli*, the infection can locate to sites other than the gastrointestinal (GI) tract.[8]

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

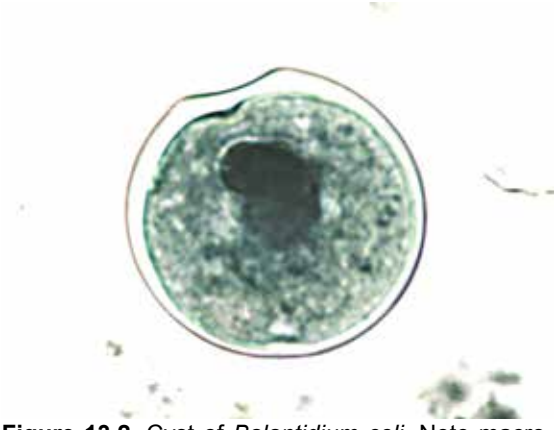


Figure 13.2. Cyst of *Balantidium coli*. Note macronucleus. 65 μm.

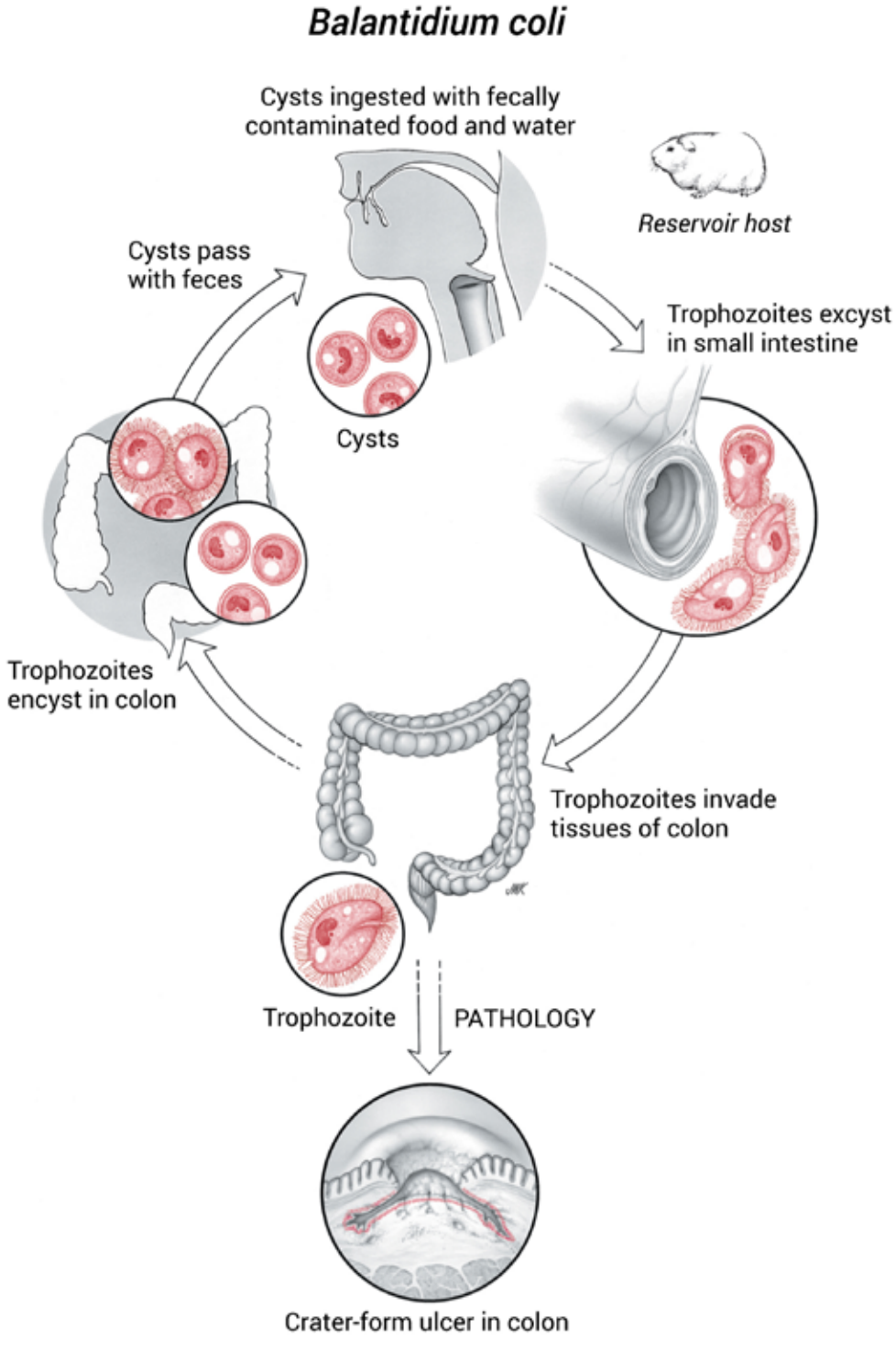
Historical Information

In 1857, Pehr Malmsten described in detail *B. coli* organisms in two patients from Stockholm, Sweden suffering from acute diarrheal disease.[9] One patient went on to recover, while the other succumbed to the infection. In 1861, Rudolph Leuckart described this same organism that he isolated from the intestine of a pig.[7] F. Stein equated these two isolates, and they were both named *B. coli*.[7, 10]

Life Cycle

There are two stages produced by *B. coli*: 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 histolytica* (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. While the cyst of *Entamoeba histolytica* is only 10–20 μm in diameter and is often present in loose stools, the cyst stage of *B. coli* measures 65 μm in diameter and is usually only seen in formed stools.[7]

Infection begins by ingestion of the cyst, in fecally contaminated food or beverages. The


Balantidium coli
Cysts ingested with fecally contaminated food and water
Reservoir host
Cysts pass with feces
Trophozoites excyst in small intestine
Cysts
Trophozoites encyst in colon
Trophozoites invade tissues of colon
Trophozoite
PATHOLOGY
Crater-form ulcer in colon

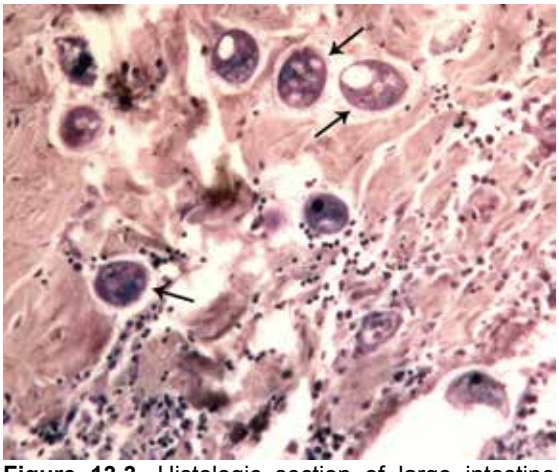


Figure 13.3. Histologic section of large intestine infected with *B. coli* (arrows).

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 liver abscess has been reported, as well as infection in the lungs and heart.[6, 11, 12] 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 type of sexual reproduction similar to conjugation.[13]

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 the requirement for an intermediate host, allowing for direct human-to-human transmission. In many ecological settings, pigs are the presumed reservoirs, since infection is more common where pigs live in close association with human habitats. Guinea pigs are thought to harbor *B. coli* as a commensal, and have been the source of some human infections.[6]

Cellular and Molecular Pathogenesis

B. coli survives in anaerobic as well as aerobic conditions and uses carbohydrates as its main source of energy.[7] The trophozoite possesses a hyaluronidase which is presumed to dissolve extracellular matrix components and disrupt the mucosal epithelial cells.[14] Proteases, most likely lysosomal in origin, released within food vacuoles (phagolysosomes), participate in the process of digestion of cell debris that enters through the peristome (mouth-like opening) that is 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.[4, 12, 15-17] Although it appears that most exposed individuals remain asymptomatic, a minority will go on to develop acute balantidiasis with watery diarrhea or dysentery.[12] Fever, nausea, vomiting, and asthenia (physical weakness or lack of energy) have been described, and there have been some deaths associated with intestinal perforation and consequent sepsis.[16] Chronic infection has been studied in endemic areas and may be responsible for negative impacts on growth in infected children.[18] Rarely, *B. coli* causes ulcerative and granulomatous disease in the colon and appendix leading to typhlitis (inflammation of the cecum) and appendicitis.[19] Individuals who are immune compromised due to malnutrition, HIV infection or from other causes may develop invasive disease with organisms invading, the lungs, urinary tract, liver and heart.[6, 8, 11, 12, 20]

Diagnosis (see Clinical Appendix)

Definitive diagnosis is by identifying the organism (trophozoite or cyst; Figs 13.1, 13.2) by microscopy in a sample of stool, or on a stained section of tissue from a biopsy of an ulcer identified by colonoscopy.[21] One might find trophozoites in freshly obtained watery stool, while only the cyst stage is present in formed stools. There are no established diagnostic serological or molecular tests available.[22] Culture is also not routinely used for diagnosis, so microscopy on stool specimens is the only diagnostic test.[22]

Treatment (see Clinical Appendix)

Although there are limited numbers of studies to guide therapy, tetracycline, metronidazole, iodoquinol, paromomycin, nitazoxanide and chloroquine have been used to treat balantidiasis.[7, 21, 23] 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 concentration of chlorine used to purify water is not adequate to destroy the cyst stage of *B. coli*.[7] 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, as is the case in many parts of the less-developed world, the risk of infection is high.[24, 25]

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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 the majority of human infections. This is a disease that not only impacts human health, but also that of domestic cattle. As an 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] A number of species that infect dogs and cats may, in some instances, be responsible for human infection.[3, 4] The occurrence of babesiosis in humans was considered a rarity, yet 139 cases were reported from New York State between 1982 and 1993.[5] Currently babesiosis is as prevalent in some areas as Lyme disease, and consequently is now classified as an emerging infection by the Centers for Disease Control (CDC).[6]

B. divergens is the most frequently occurring species in Europe, and is also considered an emerging infection in that region of the world.[7] Many species overlap in their geographic distribution, but most have discrete ecological niches, largely 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.[8] 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. Thus, making this infectious agent the first one shown to be transmitted by the bite of an arthropod.[9] Their finding inspired others to look for additional vector-borne diseases. Shortly thereafter, 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.[10] In 1969, a case of babesiosis was identified in an immunocompetent individual living on Nantucket Island, a small island off the coast of Massachusetts in the United States.[11] After additional cases were reported this became known in some areas as "Nantucket fever".[6]

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 main vector for *B. divergens* in Europe. Because *Babesia* spp. infects red cells, infections can also be acquired by blood transfusion.[12]

Infection begins by 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 vacuole.[13-16] There the sporozoite grows and

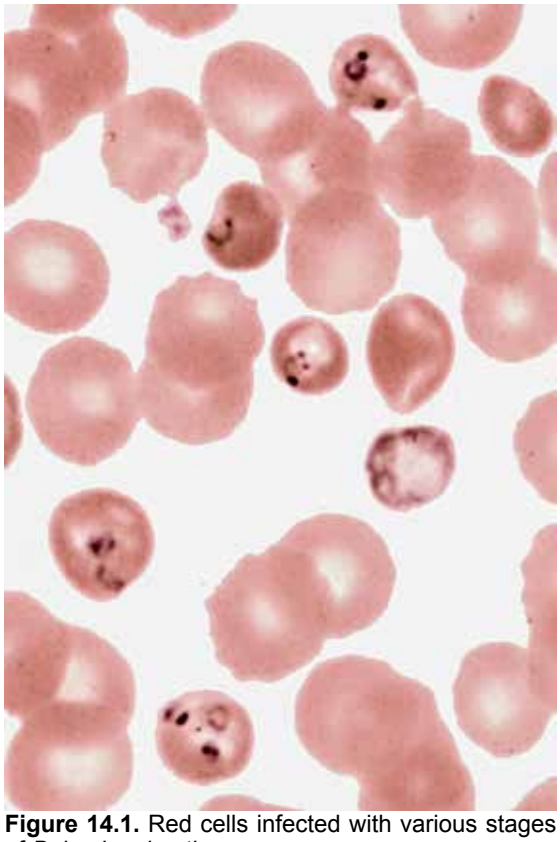

Figure 14.1. Red cells infected with various stages of *Babesia microti*.

develops, maturing into merozoites that then divide. The parasitophorous vacuole then breaks down, leaving the organism bathed in the naked red cell cytosol. It is inferred from 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.[17-19] Egress from the red cells results in 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, two weeks after the vector ingested the organisms, ookinetes develop. Ookinetes migrate to the tick salivary glands ultimately 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.[20]

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.[21-23] The self-limiting disease in immunocompetent patients is typically mild and likely often goes unnoticed without a visit to a physician. 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.[24] Disease severity appears to be dictated by the immune status of the patient, with fatality rates over 20% in some immunosuppressed populations.[19] Patients without spleens are at greatest risk of dying from babesiosis.[25-28] Patients may also be coinfected with other tick-borne pathogens, such as *Borrelia burgdorferi* (Lyme disease) and *Ehrlichia* spp. (ehrlichiosis), complicating the clinical picture.[29-34] In Europe, infection due to *B. divergens* is usually more severe.[22, 23] Intravascular hemolysis and hemoglobinuria is 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 interleukin-6 increase. These results indicate that natural killer cells may be important in modulation of infection.[35] B cells may also be important, as severe infections have devel-

oped in patients on B cell depleting therapies such as the monoclonal antibody rituximab.[36] Patients with HIV/AIDS may develop a long-term chronic infection despite specific therapy for babesiosis.[37]

Diagnosis (see Clinical Appendix)

Diagnosis is typically made by examination of Wright's or Giemsa-stained thin blood smears.[38, 39] Intraerythrocytic as well as extracellular rings can be seen and, rarely, the pathognomonic finding of tetrads of merozoites appearing as a "Maltese Cross" can be visualized on thin blood smear.[40] PCR is sensitive and specific for the diagnosis of babesiosis.[41] An ELISA-based test has been developed for both IgM as well as IgG for *B. microti,* but this test will not detect antibodies to other *Babesia* spp.[6, 42]

Treatment (see Clinical Appendix)

Combination therapy with atovaquone/azithromycin is the treatment of choice for most mild to moderate cases in immunocompetent patients, while the combination of clindamycin/quinine is recommended as an alternative.[6, 43] For severe disease, IV clindamycin combined with either oral quinine or IV quinidine is recommended.[6, 43] In some individuals, the infection may persist, even after receiving combination therapy with clindamycin and quinine.[44] Exchange transfusion is sometimes employed when patients have levels of parasitemia >10%, are multisystem compromised, or are infected with *B. divergens*.[23]

Prevention and Control

Since *Babesia* spp. infects a number of reservoir host species, some domestic and some wild, avoiding environments in which infection occurs is often difficult or undesirable. Prevention at an individual level includes checking for nymphal stage ticks (note: this stage of *Ixodes* is quite difficult to see) 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 United States, where the prevalence of *Borrelia burgdorferi* in some populations of *Ixodes* ticks have been shown to be 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.[45] Burning understory in wooded, tick-infested regions may prove useful in ecologically controlling infection in ticks.[46]

Cystoisospora belli (Formerly known as *Isospora belli*)

(Wenyon 1923)

Pronunciation: \sist-ō-ī-ˈsäs-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.[47] *C. belli* is a rare infection in immunocompetent individuals, but in recent years it has emerged as a serious diarrheal disease in patients suffering from HIV/AIDS, and in other immunosuppressed individuals.[48, 49]

Life Cycle

Infection is initiated by ingestion of the infective sporulated oocyst in fecally contaminated food or beverages. Oocysts require a period of 1–2 days outside the host in order to undergo sporulation and become infectious to a new host. Direct person-to-person transmission is not thought to occur.[50, 51] The oocyst (Figs. 14.2, 14.3) measures approximately 25 μm by 15 μm. Four sporozoites reside within

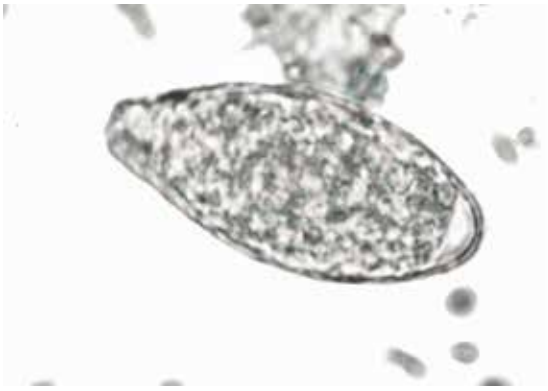

Figure 14.2. Unsporulated oocyst of *C. belli*. 20 μm.

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.[52-54]

Pathogenesis

C. belli causes a protracted, secretory diarrhea in AIDS patients with depressed $CD4^+$ counts. In patients suffering from other varieties of immunosuppression, disease resembles that induced by *Cryptosporidium*.[55-58] In most immunocompetent patient populations, infection is either subclinical or the diarrhea is transitory. Malabsorption of fats in immunocompromised patients has been reported.[59]

Figure 14.3. Sporulated oocyst of *C. belli*. 20 μm.

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.[60] Patients may die from wasting associated with protracted weight loss and electrolyte imbalance.[61] Usually symptoms resolve with specific treatment of the infection or reconstitution 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.[62-64] 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.[49] At times, duodenal aspirates are required to make the diagnosis and biopsy of small intestinal tissue in heavy infections often reveals the intracellular parasites.

Treatment (see Clinical Appendix)

Trimethoprim-sulfamethoxazole is the therapy of choice. Its use for prophylaxis in the HIV-infected population along with HAART

has effectively decreased the incidence of this pathogen in many parts of the world.[65] Pyrimethamine is an alternative therapy for infection in adults and ciprofloxacin is a second line agent that is a less effective option.[66] Although associated with some failures, nitazoxanide is another option with some efficacy in the treatment of *C. belli*.[67, 68]

Prevention and control

Avoiding fecal contamination of food, and proper disposal of human feces is the best way to prevent infection.

Cyclospora cayetanensis

(Ynes R. Ortega 1991)

Pronunciation: \sī-klō-ˈspȯr-ə\

Introduction

Cyclospora cayetanensis (\SCI-KLO-spor-ah\) causes watery diarrhea in humans and is acquired from contaminated food and water.[69-71] Although at one time this infection was virtually unknown, it has been recognized as a significant cause of diarrhea throughout the world including the United States, where approximately 15,000 cases occur per year, with some requiring hospitalization.[72, 73] In some populations, particularly in tropical regions of Peru, Brazil, and Haiti, it is endemic, causing more mild disease in children and adults, but more severe disease in immunocompromised individuals, such as those with HIV/AIDS.[74, 75]

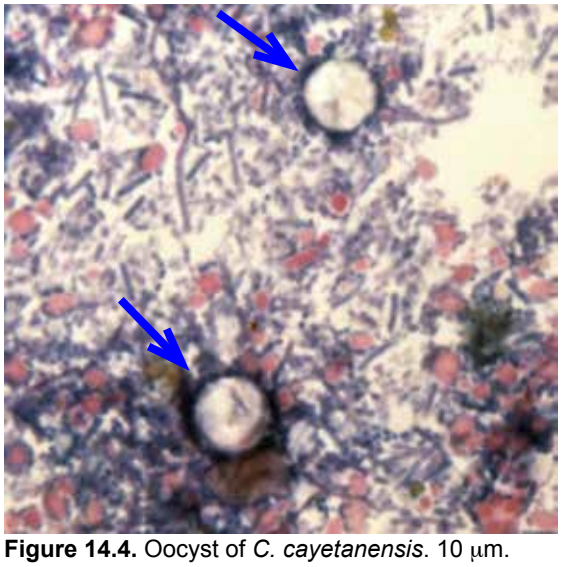

Figure 14.4. Oocyst of *C. cayetanensis*. 10 μm.

In regions of the world where this disease is endemic, epidemics tend to be seasonal, and in studies conducted in Guatemala coincided with the spring raspberry harvest.[76-78] In the United States most cases are seen in returning travelers or after ingestion of imported contaminated foods such as raspberries, basil, snow peas, and mesculin lettuce.[79-81] Several outbreaks in the United States have been caused by ingestion of fecally contaminated imported raspberries that may have come from Guatemala.[82-84]

The life cycle can be completed in a single host species, but little is known about the details of the life cycle, or the role of reservoir hosts in maintaining infection in the environment.[85-87] Studies surveying feces from multiple animals have reported the presence of *Cyclospora* oocysts in numerous animal species suggesting a role in maintaining this organism in the environment. Others report not detecting oocysts and multiple unsuccessful attempts to infect multiple types of animals.[88]

Historical Information

In 1979, Bailey Ashford described several cases of diarrhea in Papua New Guinea with a coccidian detected in the stool.[89] In 1986, Rosemary Soave reported several cases of diarrhea in returning travelers that may have been due to a coccidian pathogen.[90] In 1991, Ynes R. Ortega characterized this organism as a new coccidian species and named this organism, *C. cayetanensis,* after her *alma mater* the Peruvian University Cayetano Heredia in Lima Peru.[91]

Life Cycle

Unsporulated oocysts are released into the environment by an infected host. A period of 1–2 weeks is required before oocysts 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, then develops into the sexual stage, resulting in the production of unsporulated oocysts that are then shed into the environment.[88]

Clinical Disease

The clinical presentation and frequency of asymptomatic infected individuals varies in different parts of the world where *C. cayetanensis* is endemic. In general, infections tend to decrease in severity and duration after repeated exposures.[92] Following initial infection, watery diarrhea with 5–15 bowel movements per day ensues within a week or two, depending upon the initial number of oocysts ingested. In some cases, the onset of symptoms may be as long as 2 months after exposure.[93] In immunocompetent individuals, symptoms can last up to 2–3 weeks.[94] In those with HIV/AIDS, diarrhea can be protracted.[95] Nausea, vomiting, anorexia, and abdominal cramping are frequent symptoms during the acute phase of the infection.[86] 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.[96, 97]

Diagnosis (see Clinical Appendix)

Diagnosis can be made by NAAT, or by microscopic identification of oocysts in stool samples (Fig. 14.4). *C. cayetanensis* oocysts can be seen without staining, but the use of modified acid-fast staining techniques can improve the sensitivity up to about 30%.[95] PCR was initially introduced to monitor foods. Currently, molecular testing is in use clinically and is now included in fecal NAAT panels, such as the BioFire Film Array gastrointestinal (GI) panel.[98, 99]

Treatment, Prevention and Control (see Clinical Appendix)

The drug of choice for treatment of infection with *C. cayetanensis* is trimethoprim-sulfamethoxazole.[100, 101] Ciprofloxacin is an alternative for patients with sulfa allergies but this option may result in lower cure rates.[102] Nitazoxanide may also be an option for treatment.[103]

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-ˈglir-ē-ə\

Introduction

Several groups of free-living amoebae cause serious 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*.[104] All of these species are worldwide in distribution and have been isolated from all types of freshwater habitats and soils.

Naegleria fowleri

Pronunciation: \nā-ˈglir-ē-ə\

N. fowleri, commonly known as the brain-eating amoeba, is a robust thermophilic free-living amoeba found around the world in warm freshwater.[105] It thrives in standing freshwater environments such as hot springs, heated swimming pools, and hot tubs. The trophozoite (Fig. 14.5) measures 20–30 μm in diameter, while its cyst is smaller, measuring 8–10 μm in diameter. *N. fowleri* was found in abundance in the thermal spas built by a Roman legion in what is now Bath, England.[106] Its discovery there caused the spa to be temporarily closed, and the resulting archeological dig through the sediments that had built up over the centuries led to fascinating glimpses into the lives of this ancient army during its occupation of that region.

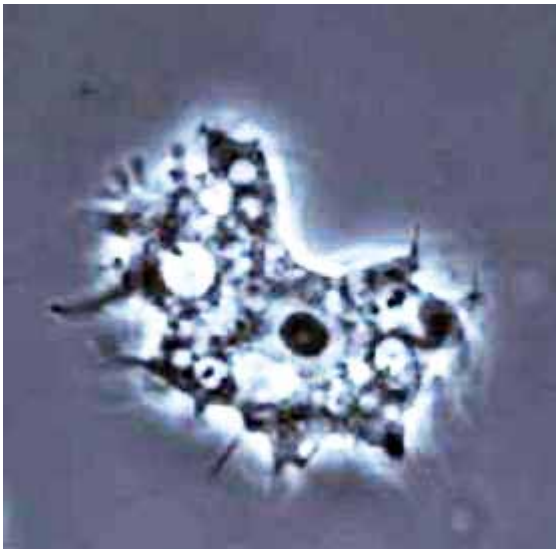

Figure 14.5. Trophozoite of *Naegleria fowleri*. 25 μm.

Clinical Disease

N. fowleri causes a serious, often fatal, fulminating infection of the CNS, referred to as primary amoebic meningoencephalitis (PAM).[107, 108] Cases of PAM have occurred in the United States, Europe, Australia, South and Central America, and Southeast Asia.

The infection is typically acquired by swimming or bathing in water above 37 °C. It is presumed that this unusual environment results in the selection of an abundance of thermally-tolerant organisms, including *N. fowleri*.[109] Diving or playful splashing activity can force heated water containing the trophozoites up into the nose and through the cribriform plate. *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 their way through tissue, probably aided by a pore-forming protein similar to that of *Entamoeba histolytica*.[110] Symptoms include severe frontal headache, vomiting, confusion, fever and coma, followed by death.[111]

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 the sensitivity of detection.[112, 113] Biopsy is another option that may reveal organisms. Diagnosis can be delayed by the fact that PAM resembles symptoms of meningitis, a more commonly occurring clinical entity. Since death often ensues within five days after the acquisition of 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 for *N. fowleri* have been developed for its detection in water samples.[114]

Treatment and Prevention (see Clinical Appendix)

Amphotericin B was the initial therapeutic agent used to treat patients, but mortality remained greater than 95% despite administration.[115] In 2013, two young children in the United States were treated with miltefosine added to their treatment regimen and both children survived.[116, 117]

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 normally found in benthic zones (the lowest levels of water), and only gain access to the water column during periods of lake "turn over."[118] There are certain behaviors such as the use of neti pots and religious ablutions where warm water, potentially containing these amoebae, is forced up the nose. Modifications of these behaviors or only using water that does not contain these amoebae could reduce infection risk.

Due to the rarity of this disease, most situations leading to infection must be classified as incidents of unlucky circumstance, especially when one considers the number of visits to hot tubs, spas and natural hot springs, and the number of user hours spent relaxing in them.

Acanthamoeba spp.

Pronunciation: \ə-ˌkanth-ə-ˈmē-bə\

Clinical Disease

Acanthamoebae (\ah-KAN-thah-MEE-bah\) are free-living amoeba associated with both keratitis and granulomatous amoebic encephalitis (GAE). *Acanthamoeba* spp. infections most commonly occur in immunocompromised patients, especially those with HIV/AIDS, but in the case of keratitis due to contaminated contact lens solutions immunocompetent individuals can be infected.[119, 120] Both the growing number of immunocompromised individuals and the increase in contact lens wearers are contributing to the rise in human cases.[121]

Acanthamoebae have both a trophozoite and a cyst stage. The trophozoite and the cyst are both approximately 13–23 μm in diameter.[122] The route of infection of *Acanthamoeba* spp. is most likely via the lungs or skin, resulting in multiple foci of infection.[123] It is probable, given the ubiquitous nature of this organism, that almost all human beings encounter them at some point during their lives. Disease, therefore, is then due to either a particular type of exposure or to a particular host susceptibility feature.[124]

Acanthamoeba spp. have the ability to invade the CNS.[125] In the brain, a slowly developing, ulcerative granulomatous disease develops, characterized by diplopia, frontal headache, seizures, and occasionally death. Patients with HIV/AIDS may experience overwhelming disseminated infection.[119, 126] Ulcerative keratitis of the eye caused by *Acanthamoeba* spp. occurs primarily in those who use contact lenses that are routinely washed in unfiltered tap water.[127] This is now considered a rare situation, primarily due to targeted public health education programs, and the availability of sterile lens cleaning solutions. 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)

Definitive diagnosis depends upon microscopically identifying the amoebae in biopsy tissue, CSF, or lachrymal secretions, or through the use of PCR.[128] A reliable staining method is available, employing Field's staining reagent.[129] 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, owing to both a lack of clinical experience and absence of controlled trials evaluating therapies.[122, 130] Few patients with GAE survive, regardless of treatment, particularly those suffering from HIV/AIDS. Keratitis is also difficult to treat, but some drugs show promise, particularly topical miconazole, propamidine and neosporin.[130] Prevention of keratitis is straightforward and simple; use only sterile contact lens cleaning solutions. These products are easily obtained at any drug store as over-the-counter preparations. In contrast, HIV/AIDS predisposes individuals to topical or inhalational entry routes, and 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.[131] Its name was derived from the late parasitologist William Balamuth and the mandrill from which it was first identified.[132] *B. mandrillaris* is found in soil, and the route of initial infection is likely via the skin, but inhalation can also occur, resulting in an initial localized infection with the potential for spread to the brain.[133] *B. mandrillaris* encephalitis can occur in immunocompetent individuals. Like *Acanthamoeba, B. mandrillaris* has both a trophozoite (15–60 μm in diameter) and a dormant cyst stage (13–30 μm in diameter).[132] Current investigations support a role for 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.[134] GAE can then develop. Most patients initially present with painless nodules and skin changes at the site of entry, followed by meningeal symptoms, such as fever, stiff neck, and headache that may progress to severe encephalitic manifestations with incomprehensible speech.[135] *B. mandrillaris* is not an infection limited to the tropics, as a large number of cases have been recognized in the United States.

Diagnosis (see Clinical Appendix)

Although *B. mandrillaris* may be seen on biopsy specimens from the skin lesions or from distal sites such as the brain, expert knowledge is required to recognize the morphological characteristics of this pathogen. Identification of an amoeba seen on microscopic evaluation can be confirmed with both NAAT and through the use of immunofluorescent antibodies specific for *B. mandrillaris.*[136] The DNA sequence of this organism is now known.[137] The CDC offer diagnostic assistance.

Treatment and Prevention (see Clinical Appendix)

This disease has been associated with a very high mortality once there is brain involvement and GAE manifests. Initial attempts at treating patients with amphotericin were associated with patient deterioration, but the use of multi-drug regimens containing 4–5 agents such as amphotericin, fluconazole, albendazole and miltefosine has resulted in some patients surviving and doing well, despite treatment initiation after the onset of GAE. Other medications such as voriconazole, flucytosine, pentamidine, azithromycin, clarithromycin, trimethoprim-sulfamethoxazole and sulfadiazine may have a role in the treatment of this pathogen.[136]

With the limited number of cases seen and no readily identifiable host susceptibility factors in most cases, 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.[138] *B. hominis* has been described in detail at the electron microscope level, and this study recognized only two stages; the vacuolar stage (Fig. 14.6) and the cyst.[139] Other investigators have described four major forms, while in reality the extensive variation in forms is just one more aspect of this organism that makes the study of its biology challenging.[140] Division is by binary fission. No sexual aspect to its life cycle has been documented. Infection is presumed to be via the fecal-oral route, and the cyst stage may be important in transmission. The cyst is small, measuring 2–5 μm in diameter, and is protected by a multilayer cyst wall.[141] It can be grown in axenic culture, permitting studies on its biochemical, genetic and biological properties.[142, 143]

Figure 14.6. *Blastocystis hominis*. 6 μm.

B. hominis is a very common finding on routine stool examination worldwide, even in asymptomatic individuals.[144] This organism is also frequently encountered with other more clinically defined pathogens, and this fact alone has made deciding on its status as pathogen, based upon 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 complete absence of all other pathogens was reported.[145]

Many genetically distinct strains of *B. hominis* have been characterized, so it is possible that some variants are pathogenic, while others are not.[146, 147] This could explain the high degree of variability in its clinical presentation.[148, 149] Patients with HIV/AIDS do not have an increased prevalence of *B. hominis* infection, nor do they appear to be any more affected by its presence than the immunocompetent population. Exceptions have been reported, in which the patient was symptomatic with

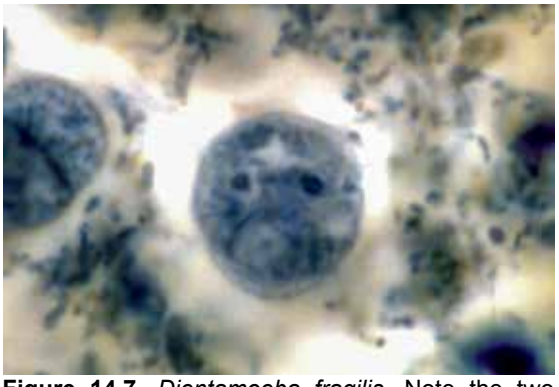

Figure 14.7. *Dientamoeba fragilis*. Note the two nuclei. 10 μm.

diarrhea and was treated successfully after diagnosis of *B. hominis* infection, only.[150] The very elderly may represent an exception, under certain as-yet-undefined conditions.[151]

Diagnosis is generally made by detection of organisms on stained smears or wet mounts of stool specimens, and PCR has been developed.[152] 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.[153] Paromomycin is another therapy suggested by some investigators to be an alternative first line therapy.[153] Trimethoprim-sulfamethoxazole has shown efficacy in the treatment of symptomatic patients and achieved high rates of *B. hominis* eradication from stool.[154] Nitazoxanide shows promise as an effective alternative therapeutic approach but studies have been confounded by its impact on other intestinal pathogens.[150]

Dientamoeba fragilis

(Jepps and Dobell 1918)

Pronunciation: \dī-ˌent-ə-ˈmē-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.[155, 156] Each trophozoite (Fig. 14.7) has 2 nuclei. For many years investigators had been unable to identify a cyst form, but cysts were finally identified both in a mouse model and in human feces.[157] 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 2 weeks.[158] *D. fragilis* is similar to *Cystoisospora belli* in that a peripheral eosinophilia can accompany infection, and patients may develop a form of eosinophilic colitis.[62, 159]

Diagnosis is either by direct examination of stool by microscopy, or by PCR, but since trophozoites are fragile and not easily detectable on wet mounts, fixed, stained stool samples are more sensitive.[160]

Many drugs have been shown to have some efficacy in treating infections.[156] Although large randomized clinical trials have yet to be carried out, metronidazole or paromomycin are recommended.[161, 162] Nitazoxanide, tetracycline, and iodoquinol are potential options for treatment of *D. fragilis* based on *in vitro* susceptibility testing.[163]

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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 a plethora of microbes whose sole purpose seems to be to colonize us and take advantage of our biochemical systems. The human body can be viewed as a series of ecological niches that select for 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 incapable of remaining on, or within, these environments and are repelled. 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]

The majority of those that take up residence do us little or no harm. In fact, by some estimates, the majority of 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, so to speak. Symbionts actively help maintain our homeostatic mechanisms. For example, the oral cavity harbors hundreds of different species of bacteria serving to exclude those that would lead 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 is devoted to a brief mention of a few of those eukaryotic organisms that we routinely harbor, and which do us no harm. The clinician may at some point receive a laboratory result with the name of one or more of these commensals on it. How these "hitchhiker" species should be approached in the context of the clinical setting is the subject of this brief chapter.

A number of commensal protozoans have been selected for life within us. Under unusual conditions, a few have been shown to be 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 a difficult time determining who did what to whom. The diagnostic microbiology laboratory now assumes a role of major importance, helping to catalogue microbes into the good, the bad, and the ugly. Resolving the primary cause of the disease often reverses the growth pattern of the opportunist. These organisms, including *Entamoeba dispar* and *E. gingivalis*, are very rarely associated with actual infection.[3-5]

It is critical for the clinician to 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-ˈmō-nəs\), *T. hominis, Enteromonas hominis* (\EN-ter-owe-MOW-nas\, \ent-ə-ˈrō-ˈmō-ˌnas\), *Retortamonas intestinalis,*

and *Chilomastix mesnili* all only colonize the human host, and are considered nonpathogenic by all standard criteria.[6] *T. tenax* lives in the oral cavity in plaque and 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 from 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 ever been linked to any abnormal health condition.

Commensal amoebae

Entamoeba dispar (\ent-a-MEE-ba\, \en-tə-ˈmē-bə\) *Entamoeba hartmanni, Entamoeba coli, Endolimax nana* (\en-DOW-lie-MAKS\, \ˈen-də-ˈlī-ˌmaks\), and *Iodamoeba bütschlii* are organisms often identified in routine stool examination. Their reporting often elicits confusion among clinicians seeking the causes for diarrheal disease in their patients. Some (e.g., *E. dispar* or *E. hartmanni)* bear a resemblance to *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. After "treatment", the illness often recurs, and drug failure is blamed. These commensal amoebae do not respond to the standard drugs used to eradicate *E. histolytica*. The use of PCR is recommended for definitive diagnosis of the 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, while *Entamoeba gingivalis* lives in the gingival flaps of a small subset of humans not yet defined, and is associated with, but does not cause, pyorrhea (periodontitis). *E. gingivalis* was diagnosed by fine needle aspiration of an abscess of the neck, following radiation therapy.[15]

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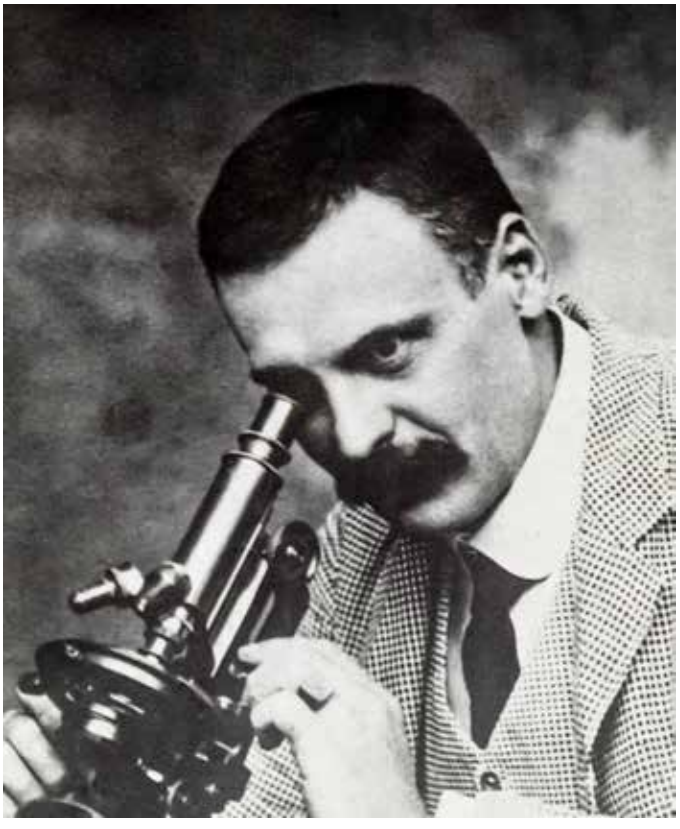


Charles Donovan, M.D. (1863–1951)

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 among the most abundant life forms on earth. The great majority of nematodes are free-living, inhabiting most essential niches in soil, fresh, and saltwater, as well as other, more specialized ones. Only a small fraction of the total number of species is parasitic, and only some of these infect the human host. Most parasitic nematodes have developed a highly specific biologic dependence on a particular species of host, and are incapable of survival in any other. Only a few have succeeded in adapting to a variety of hosts.

Best known by far among the free-living nematodes is *Caenorhabditis elegans*, whose entire genome has been sequenced (20,512 genes). In contrast, the genome of *Trichinella spiralis*, a parasitic nematode, has more total DNA than *C. elegans*, and only 60% of it is homologous with its free-living relative. There have only been 15,808 coding regions identified, implying that this parasite needs fewer, not more genes than its free-living relatives. Virulence factors, and other specialized compounds needed to resist digestion or immune attack are likely to be encoded by genes that permit the invader to live comfortably in the face of an exquisitely developed immune system.

Infections caused by nematodes are among the most prevalent, affecting nearly all of us at one time in our lives. The most common nematodes are three types of soil-transmitted helminths (STHs), the common 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. In many developing countries, children frequently harbor all three types of STHs (hence the moniker "the unholy trinity") and suffer from childhood malnutrition, physical growth retardation, and deficits in cognitive and intellectual development.

The typical nematode, both larva and adult, is surrounded by a flexible, durable outer coating, the acellular cuticle, that is resistant to chemicals. It is a complex structure composed of a variety of layers, each of which has many components, including structural proteins, enzymes, and lipids. The cuticle of each species has a unique structure and composition; it not only protects the worm but may also be involved in active transport of small molecules, including water, electrolytes, and organic compounds. A further layer, the epicuticle, surrounds the cuticle of a few parasitic species, making them even more resistant to attack from enzymes, antibodies, and other host resistance factors.

All nematodes have a well-developed muscular system. The longitudinal muscular system of nematodes lies just underneath the cuticle, and their origins and insertions are in cuticular processes. In addition, there is some muscle tissue surrounding the buccal cavity and esophageal and sub-esophageal regions of the gut tract. These muscles are particularly important elements of the feeding apparatus in both parasitic and free-living nematodes. Each muscle cell consists of filaments, 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 integration of signaling, which results in fluid, serpiginous movements. Several classes of drugs interfere only with nematode nerve signaling and are thus effective treatments for nematode infections in humans.

Nematodes have a complete, functional gut tract: the oral (i.e., buccal) cavity and esophagus, the midgut, and the hindgut with anus. The oral cavity and hindgut are usually lined by cuticle; 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, a number of specialized exocrine glands open into the lumen of the digestive tract, usually in the region of the esophagus. These glands are thought to be largely concerned with digestion, but may be related to other functions as well. For example, in hookworms, the cephalic glands secrete an anticoagulant.

In other instances, there is a single row of cells called stichocytes that 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*, for example. The function of these cells is 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 by means of the excretory system, consisting of two or more collecting tubes connected at one end to the ventral gland (a primitive kidney-like organ) and at the other end to the excretory pore.

The adult female nematode has a large portion of her body devoted to reproduction. One or two ovaries lead to the vagina by way of a tubular oviduct and uterus. A seminal receptacle for 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 to aid in transfer of sperm to the female during mating. Their identification is often based on morphology of these structures. Most nematodes lay eggs, but some are viviparous. The biology of nematodes is discussed within the text for each infectious agent as they are considered, whenever it relates to the pathogenesis of the disease.

16. *Enterobius vermicularis*
(Linnaeus 1758)

Pronunciation: \ent-ə-ˈrō-bē-əs\

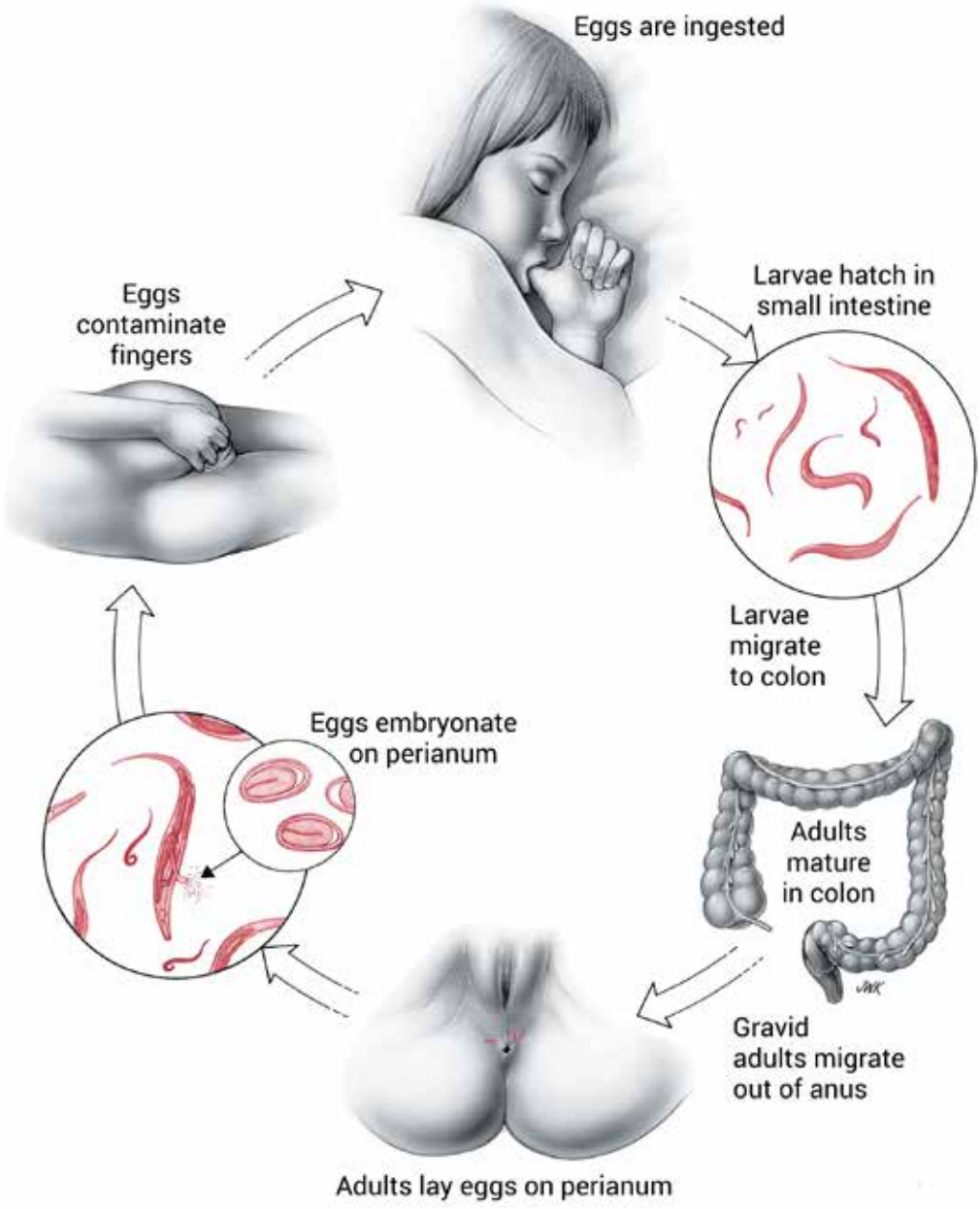
Introduction

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, pinworm still occurs with estimates indicating that it 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 communities in Europe, 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 in 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 a 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 provided an accurate description of it that forms 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

Figure 16.1. Adult female *Enterobius vermicularis.* 10mm.


Enterobius vermicularis
Eggs are ingested
Larvae hatch in small intestine
Larvae migrate to colon
Adults mature in colon
Gravid adults migrate out of anus
Adults lay eggs on perianum
Eggs embryonate on perianum
Eggs contaminate fingers

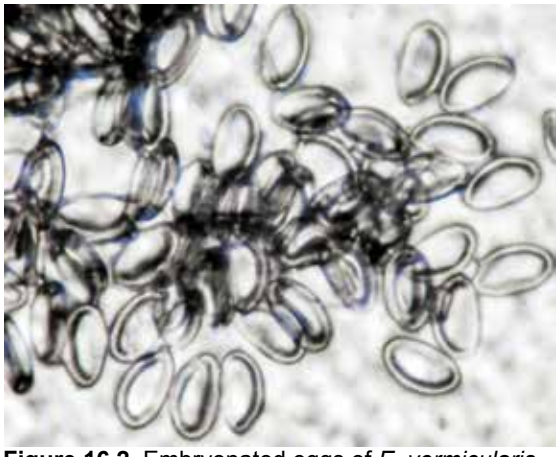


Figure 16.2. Embryonated eggs of *E. vermicularis*.

years, and *Enterobius* DNA has been detected in ancient DNA from North and South American human coprolites.[10, 11]

Life Cycle

The lifecycle of the pinworm is one of the simplest among parasites with a typical nematode pattern of development: four larval stages (L1–L4), and the adult stage. Adult worms 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 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.

The adult worms mate, and within 6 weeks, each female contains approximately 10,000 fertilized, non-embryonated eggs. Males die shortly after copulation. The gravid female migrates out the anus onto the perianal skin at night, most likely stimulated to do so by the drop in body temperature of the host. There, she experiences a prolapse of the uterus, expels all her eggs, and then dies. Expulsion can be so intense that the eggs become airborne. The eggs rapidly embryonate and become infective within 6 hours of being laid, exhibiting one of most rapid embryological developmental cycles among all nematode species.

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 these eggs can occur when a child places infective 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 and then molt, transforming into adults that travel to the large intestine and appendix (Figure 16.3), where they take up residence. The entire cycle is completed within 4–6 weeks after ingestion of the infectious egg. Alternatively, eggs can hatch on the skin at the site of deposition, 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 parasites infect the fallopian tubes. Aberrant infections also include pelvic peritonitis, ovarian infection, and granuloma of the liver.[13-18]

Cellular and Molecular Pathogenesis

All stages of *E. vermicularis* develop in the gastrointestinal (GI) tract, so the host does not experience any systemic reactions unless the worm burden is particularly high, or there is ectopic infection. The parasite elicits a mild, local inflammatory response, and while

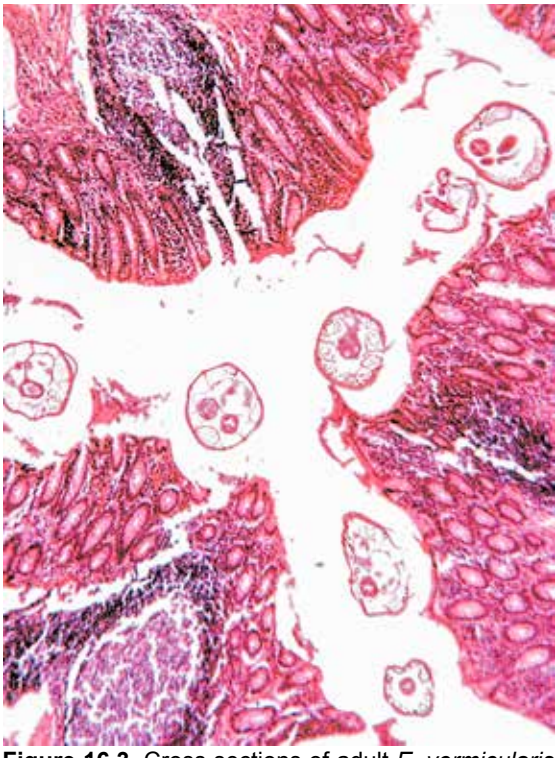


Figure 16.3. Cross sections of adult *E. vermicularis* in appendix.

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, is unclear.[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 immune status of the host affects the outcome of the infection. *Syphacia oblevata* is a pinworm species that infects mice only, and reaches much larger numbers in nude (athymic) mice than it does in the same mice into which a subcutaneous implant of thymic tissue from syngeneic donors was introduced.[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 on the basis of 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. It remains to be determined whether this difference in susceptibility has an immunological or physiological basis.

Clinical Disease

The great majority of infected individuals are free of symptoms. Those few who are symptomatic experience itching of the perianal area, which in rare instances leads to cellulitis.[22] The entire cycle is completed within 4–6 weeks after ingestion of the infectious egg. Aberrant vaginal infection leads to vaginal itching and sometimes serous discharge. Enuresis has been attributed to infection with pinworm, but no causal relation has been established.[23] Sleep disturbances have been related to the irritating perianal discomfort due to pinworm infection.[12] Patients who experience abdominal pain during infection may do so because of co-infection with *Dientamoeba fragilis* or due to another cause. Eosinophilic enteritis caused by *E. vermicularis* can be hemorrhagic and presents with abdominal pain and melena. Rarely, enterobiasis has been linked to clinical appendicitis.[16]

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 of little utility in diagnosing this infection. Eggs are best obtained by harvesting of these from the perianal area using clear (not frosted) adhesive tape or the commercially available adhesive pinworm paddle. The adhesive tape or paddle should

be applied to the perianal region in the early hours of the morning as the patient sleeps or as soon as the patient awakens (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. On occasion, thread-like female worms may be directly visible on the perianal skin. These female worms are 8–13mm long and very thin having the appearance of small white pieces of thread. Serologic tests specific for *E. vermicularis* are not used clinically for the diagnosis of this infection.

Adult pinworms can be readily identified when they are seen on histologic sections because of bilateral cuticular projections known as alae. In patients with abdominal pain or other GI symptoms, a fecal examination may be necessary to rule out co-infection with other infectious agents. 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.[24-27] 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.[24-27] Since eggs can survive 2–3 weeks on clothing, inanimate objects, and bedding, reinfection can continue to occur only for this limited period of time and thus one retreatment is usually sufficient. Knowing that the entire cycle takes 4–6 weeks after ingestion of the infectious egg can help with identification of any contacts that might be infected as well as identification of 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 prior to 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 the young child a cycle of infection and reinfection is frequent, because of the ready transmissibility of the pinworm. The groups showing highest prevalence of infection are school children and institutionalized individuals. Compounding the problem is the fact that the eggs can survive for several days under conditions of high humidity and intermediate to low temperatures. There are no predilections on the basis of sex, race, or socioeconomic class.

Thorough washing of hands with soap and water after using the toilet, changing diapers, by children or their carers 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. In institutions, daycare centers, schools, or other areas with pinworm infections, mass treatment during outbreaks can be successful.[28]

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17. *Trichuris trichiura*

(Linnaeus 1771)

Pronunciation: \trik-ˈyůr-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. The prevalence of trichuriasis is approximately 477 million worldwide, with the largest numbers of infections in Asia, Sub-Saharan Africa, and the tropical regions of the Americas.[5] It may be that trichuriasis still occurs in the southeastern region of the U. S.[1-3, 6]

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 due to *Trichuris* are usually higher in children than in adults, and disease is consequently more severe in that age group.[6] 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.[6, 7]

Historical Information

In 1740, Giovanni Morgagni was the first to describe *T. trichiura* in the cecum and transverse colon.[8] In 1761, a report by Johannes Roederer, depicted the external morphology of *T. trichiura*.[9] 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.[10] The discovery of petrified eggs in coprolites of prehistoric humans has confirmed it to be a human pathogen for over 5,000 years.[10]

Figure 17.1. Adult female *Trichuris trichiura*.

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 from ingestion of embryonated eggs to the presence of adult worms is not entirely clear. One interpretation of the data suggests that

Figure 17.2. Adult male *Trichuris trichiura*.

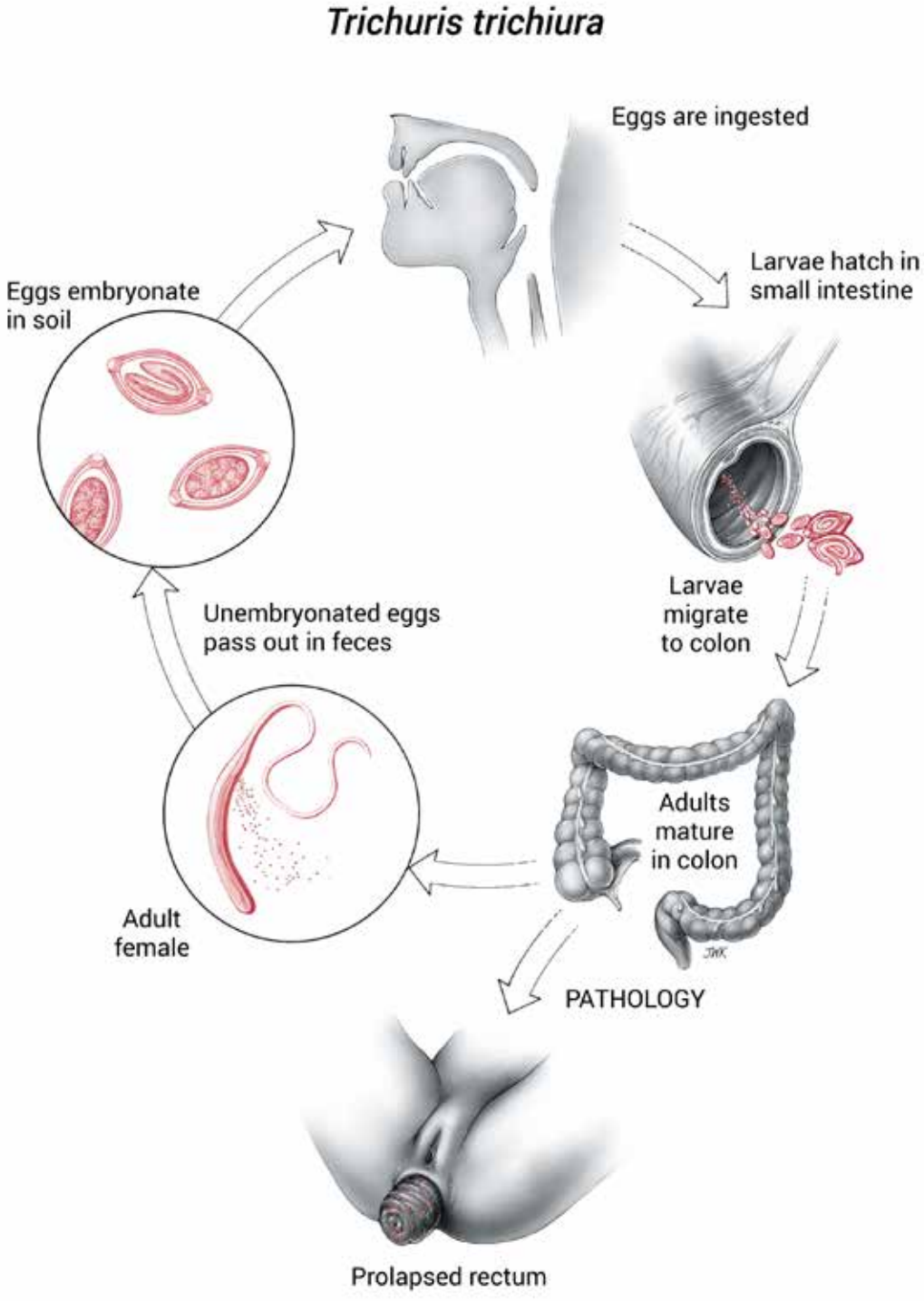
Trichuris trichiura
Eggs are ingested
Larvae hatch in small intestine
Larvae migrate to colon
Adults mature in colon
PATHOLOGY
Prolapsed rectum
Adult female
Unembryonated eggs pass out in feces
Eggs embryonate in soil

Figure 17.3. Fertilized, non-embryonated egg of *T. trichiura*. 50 μm x 20 μm.

the L1 larva hatches in the small intestine, penetrates the columnar epithelium, and comes to lie just above the lamina propria. Four molts later, the immature adult emerges and is passively carried to the large intestine, where it re-embeds itself in the columnar cells, and then induces its essential niche. Others challenge the importance or even 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 hatching of the larvae that ultimately invade the columnar cells of the colon.[11]

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 anterior, narrow, elongate esophagus of the adult worm 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.[12] 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 following the time of ingestion of embryonated eggs. Females can produce up to 3,000–5,000 eggs per day, and live for 1.5–2.0 years.[13, 14] Fertilized eggs are deposited in soil with feces, and must embryonate in the soil 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.[15] Under optimal conditions, embryonic development takes place over an 18–22 day period.[16]

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 not known 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.[17] There also appears to be a genetic component to susceptibility.[18]

The presence of adult whipworms in the large intestine induces structural defects in the

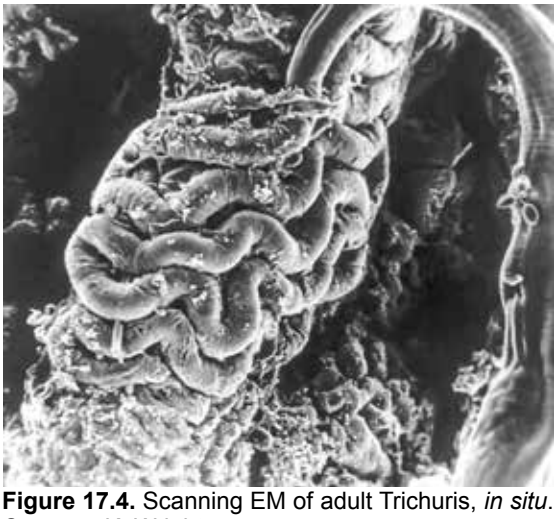

Figure 17.4. Scanning EM of adult Trichuris, *in situ*. Courtesy K. Wright.

Figure 17.5. Prolapsed rectum with adult *T. trichiura*.

epithelium.[19] In order to invade the colonic mucosa, adult *Trichuris* release a novel pore-forming and channel-forming protein.[20] *In vitro*, these secreted proteins 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.[21]

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.[22] 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.[22] Generally, the anemia resulting from heavy infection with *T. trichiura* is much less severe than hookworm-related anemia.

In contrast to situations where there is a low-grade inflammation, immunomodulatory effects of adult *Trichuris* 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* have been shown to reduce the symptoms of Crohn's disease for short periods of time without significant adverse effects on the patient.[23, 24] 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.[25, 26] Research suggests that excreted/secreted products affect intestinal epithelium, macrophage, and dendritic cells and suppress the pro-inflammatory cytokine production by these cells.[27, 28]

Clinical Disease

Clinical disease occurs mainly in children.[29] Those with very heavy *Trichuris* infections can either present with dysentery or with 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).[19]

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.[19] Following specific chemotherapy, many of these conditions 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.[7] The mechanism by which this occurs is not yet known.

Diagnosis (see Clinical Appendix)

Trichuris eggs have a characteristic appearance and are easily identified. In cases of light infection, the concentration of feces prior to microscopic examination may be required to identify the eggs. Charcot-Leyden crystals in the stool should lead to further examination even in the absence of 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 identification of *Trichuris* eggs is relatively easy, finding *Giardia* or *Entamoeba* is more difficult, and requires an experienced microscopist. Adult *Trichuris* worms can also be identified by direct visualization on colonoscopy.[30] 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 used 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 in different sections.

Treatment (see Clinical Appendix)

A benzimidazole - mebendazole or 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 for cure of trichuriasis.[31] Alternatively, *Trichuris* de-worming can sometimes be improved by adding either ivermectin or oxantel.[32] In Africa, it is currently common to combine albendazole with ivermectin in programs that simultaneously target intestinal helminth infections including trichuriasis and lymphatic filariasis or onchocerciasis.[33]

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 in children with asymptomatic trichuriasis, albendazole resulted in impaired growth, although this observation has not been confirmed in other studies.[29]

Mebendazole and albendazole are teratogenic and embryotoxic in pregnant laboratory rats at doses of 10 mg/kg. In view of these findings, the World Health Organization recommends use of these drugs in pregnancy only after the first trimester and when the benefits of de-worming 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 the age of two.[34] 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 in certain communities over 90% of the population is infected.[35] 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 in excess of 40 °C for 1 hour kills the embryo inside the egg. Eggs are relatively resistant to chemical disinfectants and can survive for protracted periods of time in raw or treated sewage. Proper disposal of feces is the primary means of prevention. In areas of the world where untreated human feces are used to fertilize crops, thermophilic composting may be a relatively low tech approach to destroying these helminth eggs.[36]

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.[6, 37, 38] 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) in order 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 theoretically interrupt transmission. High rates of post-treatment STH re-infection require that children must be treated at least on an annual basis. While there are clear health and educational benefits for school-based intervention, there are concerns that single doses of albendazole or mebendazole are often not sufficient to cure *Trichuris* infections, so that the addition of ivermectin or oxantel may be warranted.

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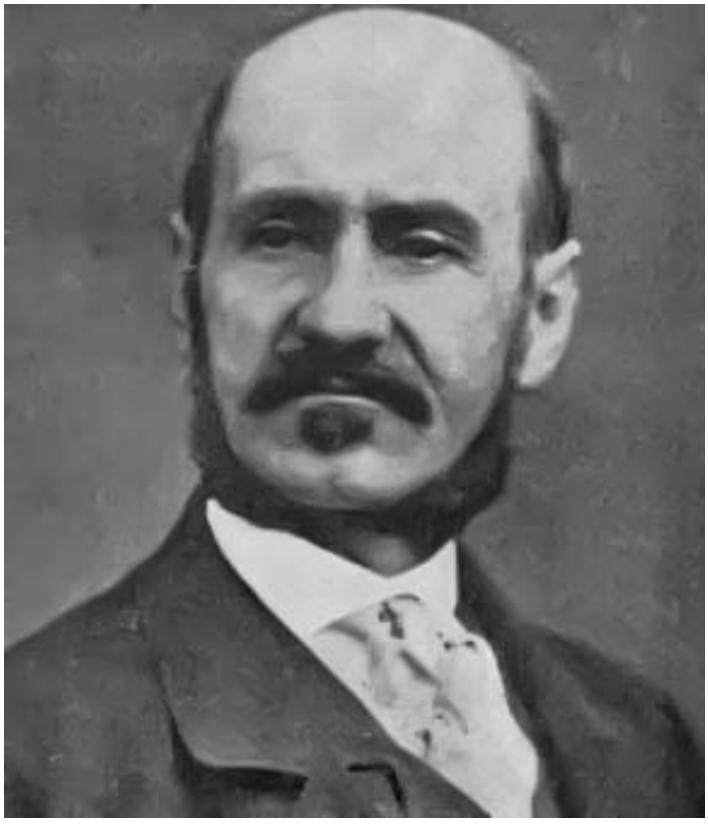


Angelo Dubini, M.D. (1813–1902)

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-kȯi-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 800 million people are infected.[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 who are 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 a variety of environmental conditions. The eggs can survive in sub-arctic regions.[4] In some developing countries, *Ascaris* eggs are ubiquitous, and have been recovered from a wide variety of environmental 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.

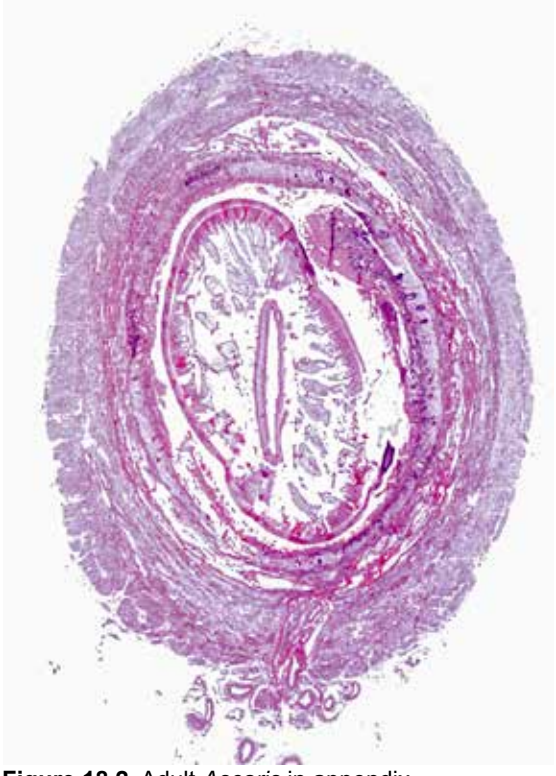


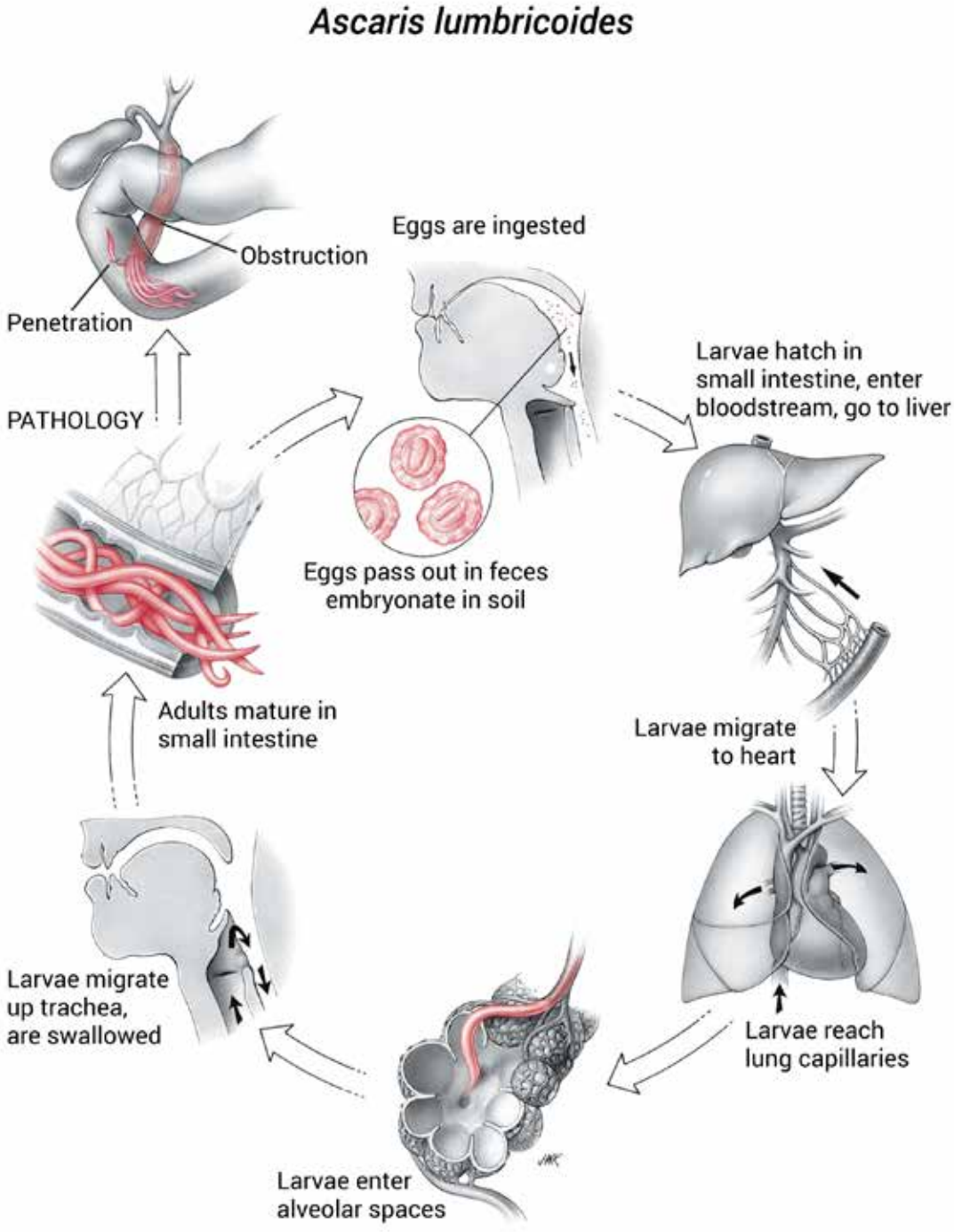
Figure 18.2. Adult *Ascaris* in appendix.

Figure 18.1. Adult female (upper) and male (lower) *Ascaris lumbricoides*. 13–18 cm in length.

It is controversial as to whether pigs can serve as an animal reservoir for *A. lumbricoides*, or whether the related parasite, *Ascaris suum* (\SO-om\, \sù-ům\), is also transmissible to humans.[7] It has been suggested that human infection arose in association with pig domestication, possibly in China.[8] The available evidence suggests that *Ascaris* in humans and pigs comprise reproductively isolated populations, suggesting that zoonotic transmission is not common.[8]

Historical Information

In 1683, Edward Tyson described the anatomy of *A. lumbricoides*, then known as *Lumbricus teres*.[9] Carl Linnaeus gave it its current name on the basis of its remarkable similarity to the earth-worm, *Lumbricus terrestrias*, which


Ascaris lumbricoides
Obstruction
Penetration
PATHOLOGY
Eggs are ingested
Eggs pass out in feces
embryonate in soil
Larvae hatch in
small intestine, enter
bloodstream, go to liver
Larvae migrate
to heart
Larvae reach
lung capillaries
Larvae enter
alveolar spaces
Larvae migrate
up trachea,
are swallowed
Adults mature in
small intestine

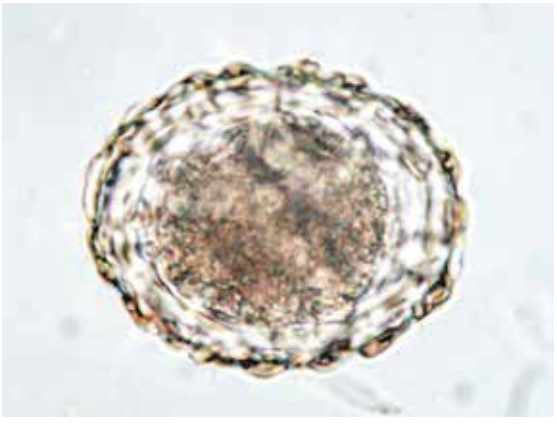


Figure 18.3. Fertilized, unembryonated egg of *A. lumbricoides*. 60 μm x 40 μm.

he also named.[10] The worm's life cycle was accurately described by Brayton Ransom.[11] In 1922, Shimesu Koino reported on a series of experiments, in which he infected both 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) occupy the lumen of the upper small intestine, where they live off predigested food, or chyme, as well as host cellular debris. The worms maintain themselves in the lumen of the small intestine by assuming an S-shaped configuration, pressing their cuticular surfaces against the columnar epithelium of the intestine, and continually moving against the peristalsis. The worms are covered with a tough, thick cuticle composed of collagens and unusual lipids, enabling them to successfully resist being digested by hydrolases. The adult worms produce a battery of protease inhibitors, some of which may also interfere with host digestion.

The egg production of the adult female worm is prolific, producing, on average, 200,000 eggs per day, each of which can survive up to 10 years in the right conditions.[13] The uterus of the adult female worm may contain up to 27 million eggs at any one time. To synthesize the 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 actually serve to 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 they 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 takes place 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 inner layer of the egg, and may confer a number 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. Embryonated eggs are the infective stage and must be swallowed by a host for the life cycle to continue. The L1 larva develops into the L2 larva inside the egg, but 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] The egg is then stimulated to hatch by a combination of alkaline conditions in the small intestine, and the solubilization of certain outer layers of the eggshell, facilitated by bile salts. These conditions induce a worm-specific proteolytic enzyme, facilitating hatching. The egg protease is activated by alkaline conditions, insuring that it will hatch in the right anatomic location inside the host. The infectious process is accompanied by a dramatic shift in *Ascaris* metabolism from aerobic to anaerobic.[19]

The immature L3 parasite, now in the 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 into the pulmonary circulation. The larva molts once more and grows larger, both in length and in diameter. It becomes stuck in an alveolar capillary, since its diameter is now much greater 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 "*verminous*" pneumonia.

Figure 18.4. Larvae of *A. lumbricoides* in liver of experimentally infected mouse.

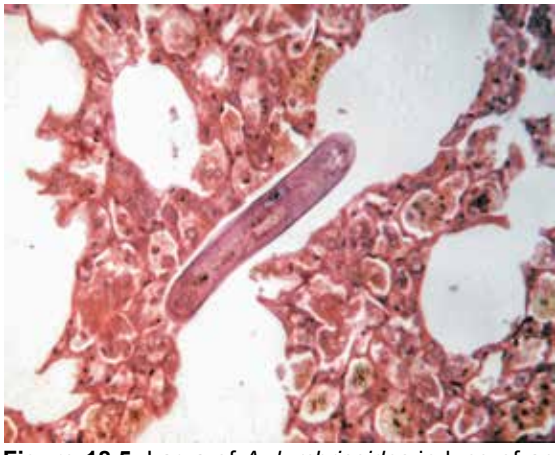


Figure 18.5. Larva of *A. lumbricoides* in lung of an experimentally infected mouse.

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. There, after an additional molt, the worms grow prodigiously, maturing to adulthood in about 6 weeks. Adult worms then mate. Occasionally, egg production may precede mating. When this occurs, the worm releases infertile eggs. Rarely, a single female worm is acquired, also 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 the inflammation associated with eosinophilic infiltration of the tissues, peripheral eosinophilia, and an antibody response, leading to an increase in 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 a

number of hypotheses to explain the impact of helminth infections such as ascariasis on the atopic state of the host. Among them is the notion that atopy evolved as an adaptive mechanism to promote 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 it is absorbed by the host. 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 *Ascaris*-infected children can develop malabsorption of fat, protein and vitamin A, 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 for this. There have been a number of longitudinal studies in Asia and Africa comparing 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 some of these studies, children who were treated 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 they are irritated (high fevers, drugs, etc.), which may lead them to perforate the intestine, penetrate the liver, obstruct the biliary tract, or cause peritonitis. Individuals with mild and moderate infections are rarely symptomatic. Most commonly, these individuals become aware of the infection through examination of the stools for another reason, because of passage of an adult worm in the stool, or by regurgitation of it during an episode of vomiting. Heavy infections may lead to the formation of a large bolus of adults that

obstructs 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, India determined that 0.5% of the adults in this region had evidence of hepatobiliary ascariasis.[31]

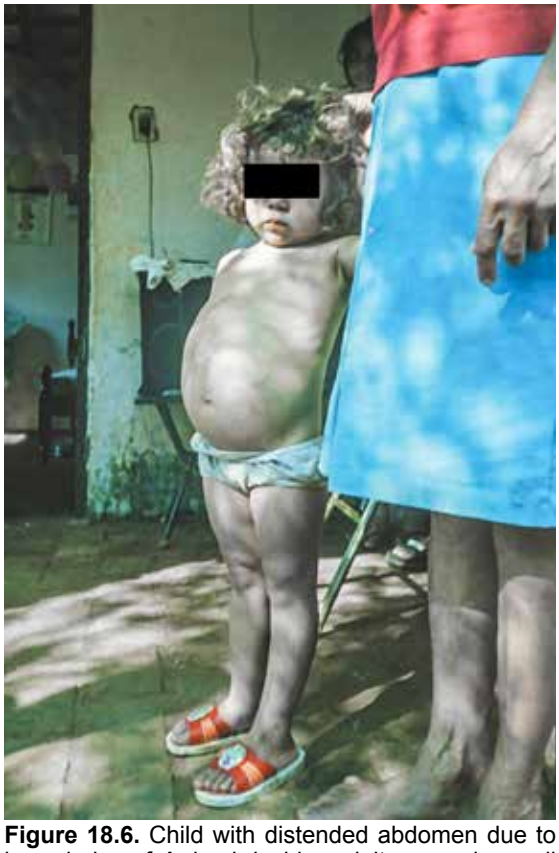


Figure 18.6. Child with distended abdomen due to large bolus of *A. lumbricoides* adult worms in small intestines.

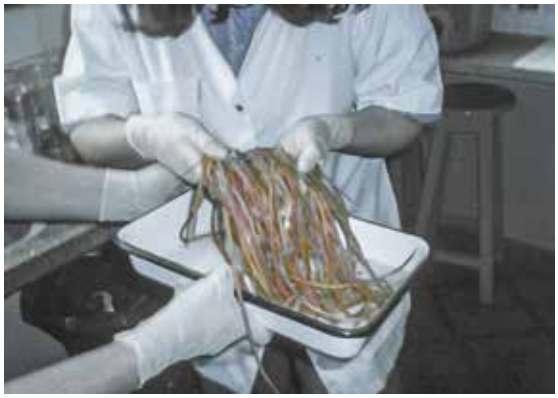


Figure 18.7. Adult *Ascaris* recovered from child in Fig. 18.6. after treatment with mebendazole.

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 not known.

Diagnosis (see Clinical Appendix)

A. lumbricoides infection cannot be specifically diagnosed solely on the basis of 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 any of 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 the presence of even a single female worm may have serious clinical consequences if she migrates. Occasionally, defective eggs missing the outer mamillations (nipplelike projections) are 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] While available for other helminths, antigen tests are not available for *A. lumbricoides*, but molecular tests (NAATs) have been developed with sensitivities high enough to detect a single *Ascaris* egg.[38]

Treatment (see Clinical Appendix)

Albendazole and mebendazole are the treatments of choice for ascariasis.[39, 40] For school-based de-worming programs, usually a single dose of albendazole 400 mg or mebendazole 500 mg is 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, although 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 a large number of worms result in an intestinal or biliary obstruction. These conditions often present 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 all but absent. Because *Ascaris* worms are hardy, it is not uncommon to also 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 at one time 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 infections. This observation, along with the health and educational benefits of de-worming, led the 2001 World Health Assembly to recommend the use of single-dose treatments of children with albendazole or mebendazole as a cornerstone of a global de-worming program. Through such programs of mass drug administration, 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 when exposed to temperatures in excess of 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 many months to years.

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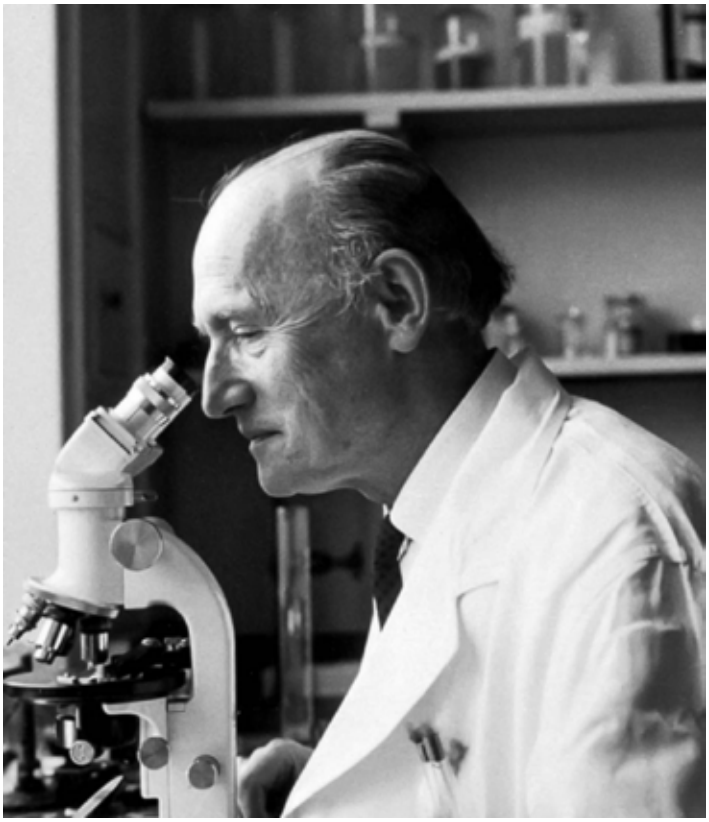


Percy Cyril Claude Garnham, M.D. (1901–1994)

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-ˈkāt-ər\

Ancylostoma duodenale
(Dubini 1843)

Pronunciation: \an-sə-ˈläs-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 by far the more common human hookworm 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, as well as anemia. Hookworms infect close to 500 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.[4-6] According to some estimates, hookworm ranks with schistosomiasis as the leading helminth infection in terms of deaths and disability-adjusted life years (DALYs) lost than any other human helminth infection.[1, 7]

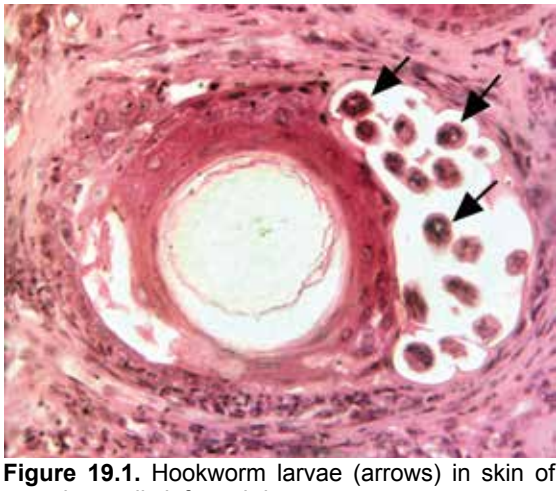
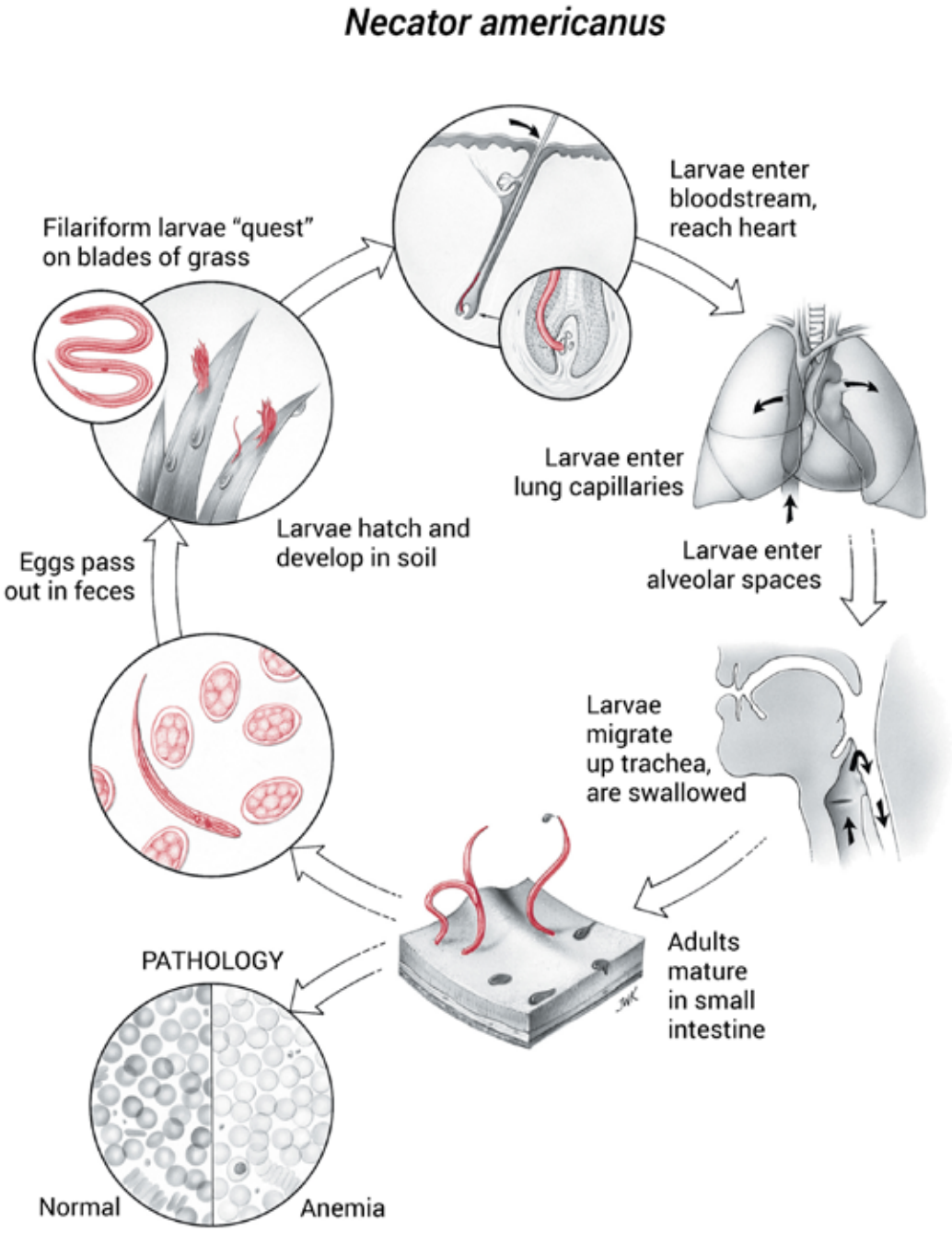


Figure 19.1. Hookworm larvae (arrows) in skin of experimentally infected dog.

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, 7-9] Hookworm is an important health threat for women of reproductive age. It is estimated that more than 40 million pregnant women are infected with hookworms in endemic countries.[5] The resulting iron deficiency and malnutrition during pregnancy adversely affect intrauterine growth, birth weight, and even maternal survival.[9] Hookworm also increases the likelihood of premature birth, and may contribute to maternal mortality.[10]

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. There are no known reservoirs for *A. duodenale* or *N. americanus*.

A. ceylanicum, is found mainly in cats, but infects humans living in Malaysia and elsewhere in Asia.[11-15] The dog is the primary host for *A. caninum*, but it has not been 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.[16] *A. braziliense*, whose definitive hosts are dogs and cats, causes cutaneous larva migrans.[17]


Necator americanus
Larvae enter bloodstream, reach heart
Filariform larvae "quest" on blades of grass
Larvae enter lung capillaries
Larvae hatch and develop in soil
Larvae enter alveolar spaces
Eggs pass out in feces
Larvae migrate up trachea, are swallowed
Adults mature in small intestine
PATHOLOGY
Normal
Anemia

Historical Information

N. americanus likely originated in Asia, while *A. duodenale* most likely originally came from Africa.[18] Hookworms appear to have been infecting humans for thousands of years in the Old World, while controversy exists as to whether hookworm was present in the Americas prior to European exploration and colonization.[18, 19] Angelo Dubini first reported human infection with *A. duodenale* in 1843; but it was Arthur Looss, working in Egypt, who demonstrated percutaneous transmission of hookworm infection and clarified its life cycle.[20] The life cycle was further defined by Gerald Schad, who demonstrated the ability of *A. duodenale* larvae to remain in a dormant arrested state in human tissues.[21]

Hookworm infection was common in the United States in the past (primarily in the rural South), as well as in Puerto Rico.[22, 23] Because hookworm disease 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.[23] 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.[24]

The prevalence of hookworm infection in the United States has been reduced almost to the point of eradication, but this resulted less from any planned intervention than from the general improvement in socioeconomic conditions. Economic development also accounts in a large part for the control of 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."[25]

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 area of skin. Skin invasion may be facilitated by the release of hydrolytic enzymes. Once in the subcutaneous tissues, larvae enter capillaries and are carried passively through the bloodstream to the capillaries of the lungs. The L3 larvae break out of the alveolar capillaries and complete the migratory phase of the life cycle by crawling up the bronchi and trachea, over the epiglottis, and into the pharynx. They are swallowed and proceed into the stomach. This portion of the life cycle (i.e., parenteral phase) closely parallels those of *Ascaris lumbricoides* and *Strongyloides stercoralis*. Two molts take place in the small intestine, resulting in the development of an adult worm (Fig. 19.2, 19.3).

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.

A. duodenale larvae are also infective orally.[26] In some regions, oral ingestion may be the predominant mode of transmission. 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.[27]

Forty days following maturation and copulation, the female worms begin laying eggs (Fig. 19.4), completing the life cycle. In some cases of infection with *A. duodenale*, larvae may stay longer in tissues before transiting to the intestine, with a resultant delay in egg production.[28] Adult worms live an average of one year in the case of *A. duodenale* and 3–5 years in the case of *N. americanus*.[29] The maximum recorded survival time is 15 years.[30]

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.[21] 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. When these larvae appear in breast milk, vertical transmission of *A. duodenale* infection in neonates may result.[31]

The adult worms feed on intestinal villi and blood in the small intestine (Fig. 19.5). Morphologically, each species can be differentiated on the basis of 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 the presence of a 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 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 are passed. In warm, moist, sandy, or loamy soil the embryo develops to the L1 (rhabditiform) larva within 48 hours of deposition in the soil. After hatching, the larva feeds on debris in the immediate surroundings, grows,

Figure 19.4. Fertilized, embryonated hookworm egg. 65 μm x 40 μm.

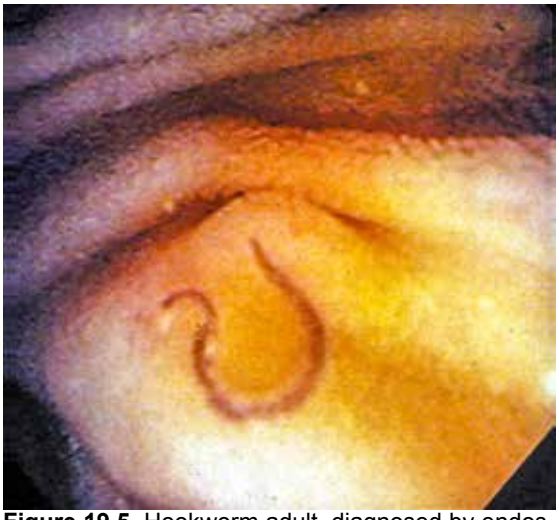


Figure 19.5. Hookworm adult, diagnosed by endoscopy.

then molts twice to develop into the infective L3 larva. Filariform larvae do not consume food, and are generally considered to be in a developmentally arrested state.[32] These larvae do not lie motionless; rather, they 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 soils, such as those found in coastal areas, are particularly favorable for hookworm larval migrations and hookworm transmission.

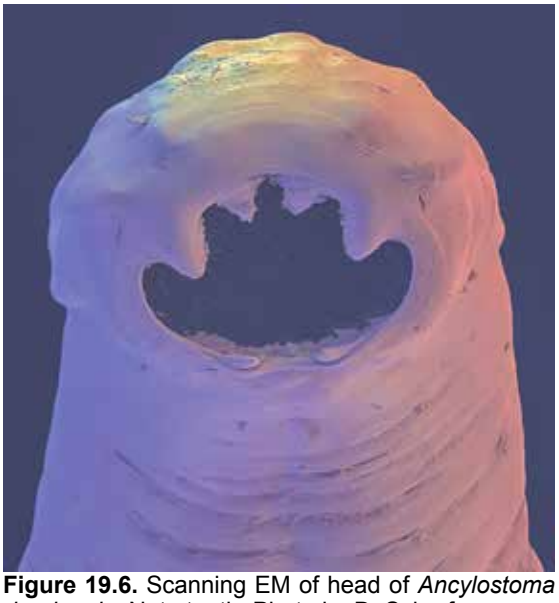


Figure 19.6. Scanning EM of head of *Ancylostoma duodenale*. Note teeth. Photo by D. Scharf.

Figure 19.7. Scanning EM of head of *Necator americanus.* Note cutting plates. Photo by D. Scharf.

Cellular and Molecular Pathogenesis

The L3 hookworm larvae penetrate unbroken skin with the aid of secreted enzymes that include a metalloprotease and a family of cysteine-rich secretory proteins, known as the *Ancylostoma* secreted proteins.[33, 34] Repeated infection results in an immediate hypersensitivity and other inflammatory responses comprising hookworm dermatitis. Subsequent larval migration through the lungs may result in pulmonary inflammation, resulting in a 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 site of attachment 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.[35, 36] Hookworms also secrete a protein that functions as a platelet inhibitor through blockade of GPIIb/IIIa.[37-39] Blood loss continues 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 per day, while each *N. americanus* adult worm sucks 0.01–0.02 ml of blood per day.[40] Following blood ingestion, the adult hookworms rupture the red blood cells with the aid of hemolysins, and then break down host hemoglobin in an ordered manner through the activities of hemoglobin-specific proteases.[41,42]

In addition to blood loss, there can be protein loss that contributes to protein malnutrition, a problem that persists in the low socioeconomic conditions where hookworm infection is still endemic.[43] Production of a hookworm serine protease inhibitor that interferes with pancreatic enzyme-based digestion may also interfere with breakdown of ingested foods, leading to malabsorption and consequent retardation of growth and development.[44]

Repeated exposure to hookworm infection does not necessarily result in protective immunity.[45] Although a humoral antibody response to numerous hookworm antigens can be demonstrated in naturally infected individuals, the presence of specific antibodies often does not correlate with resistance to infection.[46] Some immunoglobulins may even serve as a marker for hookworm infection.[47] The absence of effector 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.[7]

A concerning feature of hookworm infection is it may actually 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.[48-52] 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.[53] It may account for the difficulties associated with the control of hookworm and increased incidence of other infections such as malaria in infected patients.[54] The absence of resistance to hookworms may reflect the parasite's ability to escape host immunity, but some success with the development of a human hookworm vaccine suggest that an effective protective human immune response may be inducible.[55-58]

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.[59] These investigations offer some hope for the eventual development of effective vaccines against intestinal helminths.

Clinical Disease

In general, there are four potential manifestations of hookworm disease (dermatitis, pneumonia, abdominal pain, and chronic iron deficiency anemia) that are determined by the stage of the infection, the route of acquisition and the degree of worm burden.[1] Initial penetration of the skin 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.[28, 60] The tissue-migrating larval stages do induce circulating eosinophilia one to two months after exposure.[60, 61]

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.[27, 62]

Severe illness resulting from hookworm infection results only when large numbers of adult worms are present in the small intestine. In most endemic areas, hookworm infections "aggregate."[63] Anywhere from two-thirds to three-fourths of the infections in a given area are sufficiently light in terms of intensity (numbers of worms) that they are clinically silent. The clinical features of hookworm disease usually occur only in 10–30% of individuals who harbor large numbers of worms. In some regions, certain individuals might be predisposed to acquiring heavy infections on the basis of genetics or infectious exposure.[63, 64]

The major clinical feature of hookworm disease is iron-deficiency anemia, which occurs as the result of blood loss in the intestinal tract.[1, 7, 65-68] Intestinal blood loss is proportional to the number of hookworms present in the intestine, although 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.[65-68] 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.[69] 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.[31, 70] There is evidence suggesting that these neonates ingested *A. duodenale* through breast milk.[31]

Chronic hookworm anemia during childhood causes physical growth retardation, as well as deficits in child cognition and intellectual development.[1, 7-9] As with other soil-transmitted helminth infections, growth retardation is often reversible by administration of antihelmintic therapy, but intellectual and cognitive deficits cannot always be reversed by therapy.[71] Chronic hookworm anemia during pregnancy can result in prematurity or low birth weight.[9] Many of these chronic sequelae account for the tremendous impact of hookworms on maternal and child health. In its World Development Report, the World Bank cited hookworm as a leading cause of morbidity among school-aged children.[72]

Diagnosis (see Clinical Appendix)

Serological tests are not available outside of research settings and consequently diagnosis is usually 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 eggs of any of the hookworm species based purely on microscopy. Differentiation by light microscopy depends upon the examination of either larvae (Fig. 19.8), or by recovering adult worms. PCR tests have been developed that have increased sensitivity for diagnosis and allow for species differentiation, but these assays are not in routine use in most parts of the world endemic for hookworm.[73, 74]

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 compared with single dose mebendazole.[75] Failures of a single dose of mebendazole or albendazole to remove hookworms from the GI tract have been reported, and twice daily dosing for three days results in higher cure rates.[76-78] Pyrantel pamoate can be given for three days as an alternate therapy, but ivermectin has poor efficacy.[77, 79]

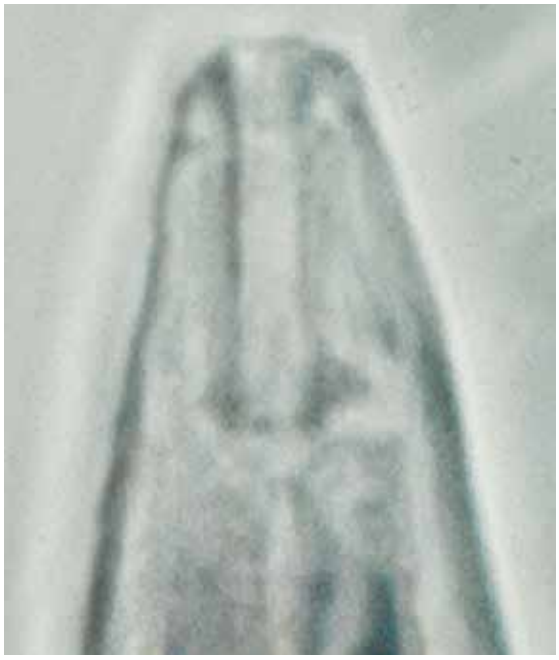


Figure 19.8. Buccal cavity of hookworm rhabditiform larva. It is longer than that of the same stage of *Strongyloides stercoralis* (see Fig. 20.6).

Very young children and pregnant women are considered especially vulnerable to the effects of hookworm anemia. In Africa, hookworm was found to make a substantial contribution to anemia in preschool children, while in Nepal hookworm was a significant cause of anemia in pregnancy.[80, 81] Pregnant women who received antenatal albendazole were shown to exhibit significant improvements in maternal anemia, birth weight, and infant mortality.[82] The concerns about the toxicities of the benzimidazoles need to be weighed against their potentially important benefits in pregnancy.[83, 84]

Albendazole and mebendazole work by binding to the microtubules of the parasite.[85] Resistance to the benzimidazoles has been well-described among nematodes of veterinary importance, and occurs via mutations in the parasite tubulin alleles.[86] 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.[76, 82] Efficacy of these drugs will need to be monitored, as they are used in mass de-worming campaigns.[82]

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.[81]

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.[87] Despite decades of widespread attempts to control hookworm through these traditional methods, the global prevalence and intensity of hookworm remains 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 during agricultural pursuits, and the continued reliance on human feces for fertilizing crops.[88]

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 was put forth as a plan to control hookworm among other diseases that involved several components including mass drug administration.[89] There are concerns that this approach might have less of an impact on hookworm than the other STH infections such as *Trichuris* and *Ascaris*. The highest hookworm intensities in a community are often not in children, so that targeting children is not expected to reduce hookworm transmission.[90] High rates of post-treatment hookworm reinfection occur in many communities, especially those with high levels of transmission, which sometimes requires thrice-yearly antihelmintic treatment.[53] 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]

As a complementing control strategy, the Human Hookworm Vaccine Initiative has developed a recombinant vaccine that targets adult hookworm blood feeding by eliciting anti-enzyme antibodies that block parasite hemoglobin digestion.[56] This vaccine is being studied for its safety and immunogenicity. Mathematical modeling of the vaccine indicates that it could reduce hookworm transmission, which would not be possible using pediatric anthelmintic drug de-worming approaches, while economic modeling indicates that a hookworm vaccine could be highly cost-effective.[91, 92]

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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-ˈlȯi-ˌdēz\

Introduction

Strongyloides stercoralis (\STRON-ji-LOI-deez\) is a parasitic nematode with a worldwide distribution. It is particularly prevalent throughout tropical and subtropical regions, as well as temperate climates. In Sub-Saharan Africa, South East Asia (e.g., Cambodia), parts of the Caribbean and in South America, prevalence rates in many areas are higher than 20%.[1] In North America, it occurs frequently among immigrants, in parts of Appalachia and in patients at long-term care 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 that 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 μm.

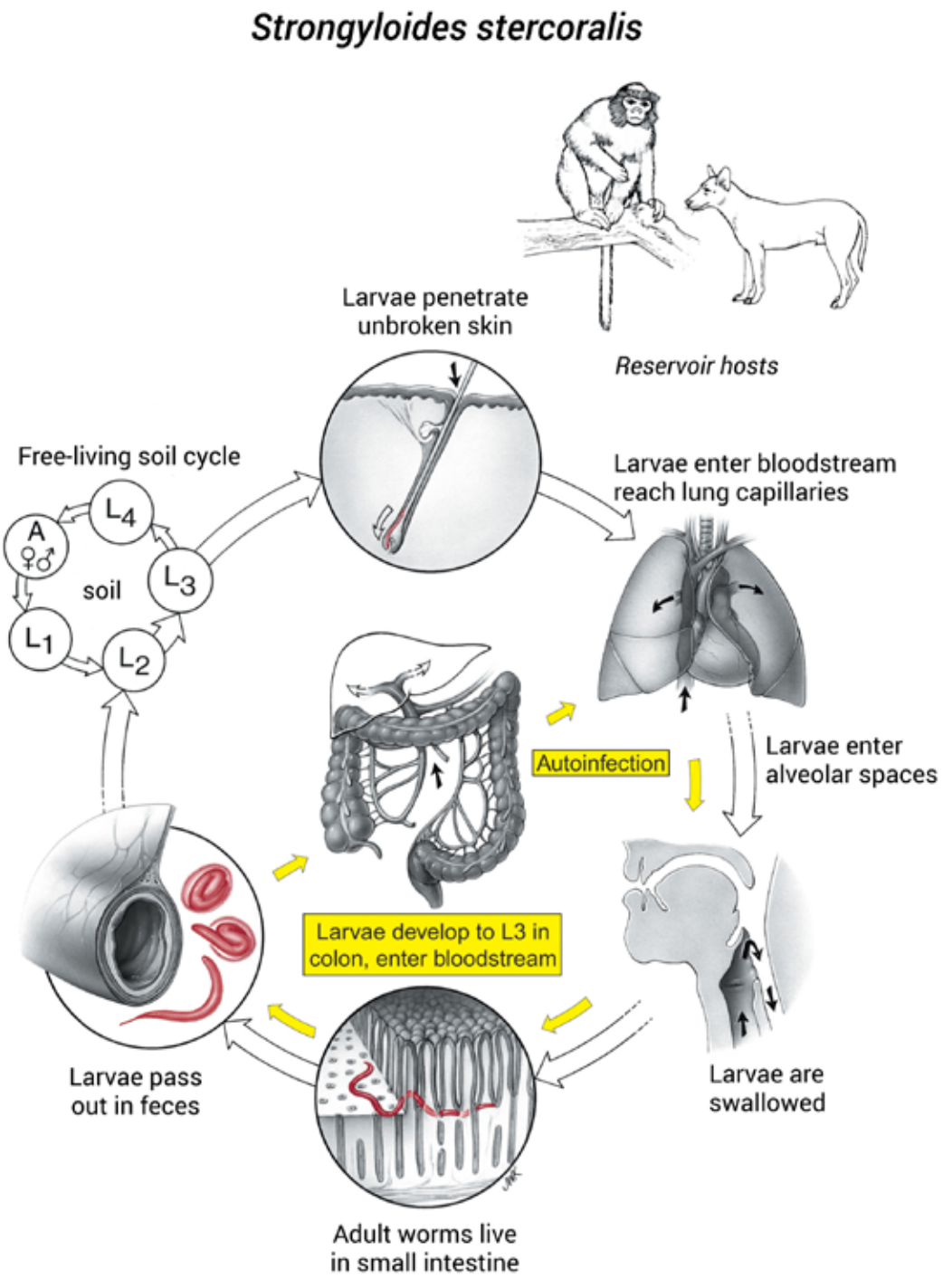
Reservoir hosts play an important part in this nematode's biology, with dogs and non-human primates able to harbor *Strongyloides* spp..[6] There have been numerous outbreaks of strongyloidiasis among dog handlers.[7] *S. stercoralis* can also reproduce as a free-living nematode in soil. In this case, the L3 (filariform) larva of that phase 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 strongyloidal 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 the life cycle was reported by Friedrich Fuelleborn.[16, 17] Fuelleborn conducted experiments in dogs and learned that infective larvae penetrate unbroken skin. In 1928, Masao Nishigoii described autoinfection with *S. stercoralis*,


Strongyloides stercoralis
Larvae penetrate unbroken skin
Reservoir hosts
Free-living soil cycle
A ♀♂
L4
L3
soil
L1
L2
Larvae enter bloodstream reach lung capillaries
Autoinfection
Larvae enter alveolar spaces
Larvae develop to L3 in colon, enter bloodstream
Larvae pass out in feces
Larvae are swallowed
Adult worms live in small intestine

and also showed that infected dogs made constipated with morphine and bismuth subnitrate passed infective 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 about 2 mm by 35 μm. The parasitic worm 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, with release of eggs into the lamina propria.[19]

Figure 20.2a. Parasitic female *S. stercoralis*. 2.5 mm x 35 μm. Courtesy L. Ash and T. Orihel.

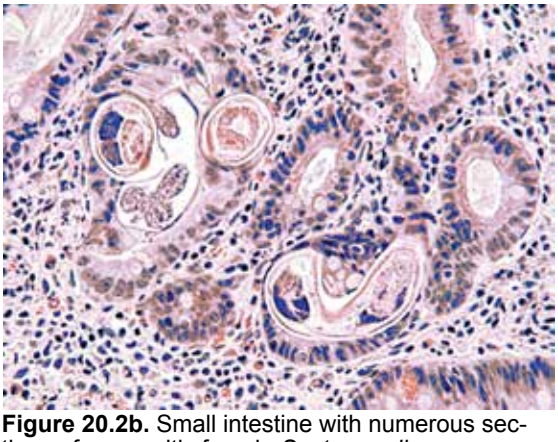


Figure 20.2b. Small intestine with numerous sections of a parasitic female *S. stercoralis.*

The embryonated eggs hatch rapidly 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 lumen of the colon, 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. In the proper soil, and under optimal environmental conditions, they develop to free-living adult worms. 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. When they mate the female produces embryonated eggs that hatch as L1 larvae and develop into L2 larvae that then molt to become L3 larvae.

The L3 larva can infect humans and other susceptible hosts. When conditions become

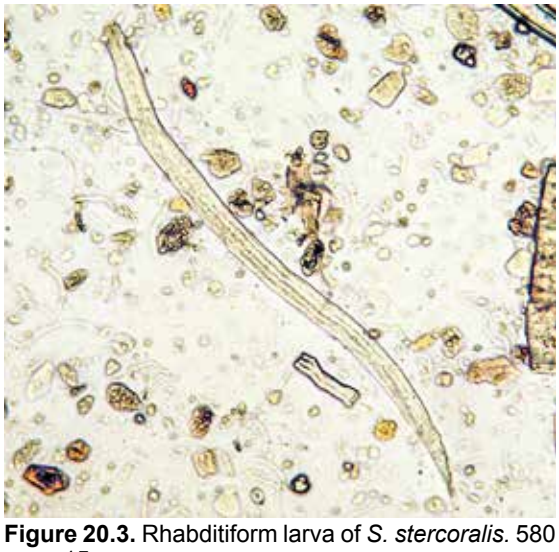


Figure 20.3. Rhabditiform larva of *S. stercoralis*. 580 μm x 15 μm.

unfavorable for the continuation of the free-living phase (e.g., lack of nutrition, low moisture) more L2 larvae transform into the infective L3 stage that can remain in 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 in which to find a host, as compared to hookworm L3 larvae, which cannot do so. The free-living life cycle can only occur for one generation with *S. stercoralis* as after that the nematode must enter a host for further cycles to occur.

Parasitic Phase (Homogonic Life Cycle)

The L3 larva enters the host through the skin, a process facilitated by the release of a protease 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. 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. *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 and becomes the parasitic parthenogenic female. Egg production begins within 25–30 days after the initial infection.

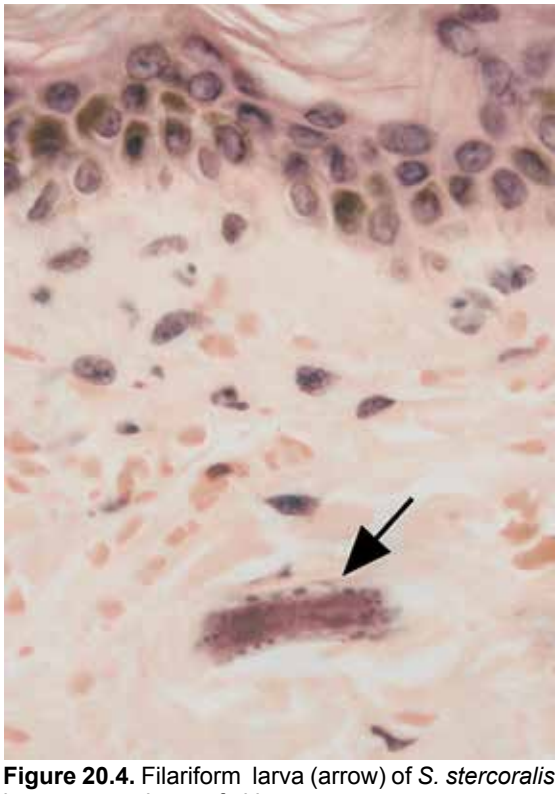


Figure 20.4. Filariform larva (arrow) of *S. stercoralis* in cutaneous layer of skin.

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 referred to as 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, although 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 and 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 mucosa of the small intestine but do 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 cases of hyperinfection or disseminated infection, 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 tissues of the host, 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. The majority of infected patients display no symptoms following infection, and peripheral eosinophilia may be the only evidence of acute infection.[41, 42] Some individuals may report skin reactions, commonly called ground itch, due to penetration of the skin by the infecting L3 larvae.[43] The rash of ground itch is characteristically on the feet. In the 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 for 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 antihel-

mintic 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 as a result of 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. 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 the context of many immunosuppressive conditions, with many immunosuppressive agents, and with 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 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 intestine. These patients often have secondary bacterial enterocolitis that can result in a paralytic ileus, and bacterial invasion that results in metastatic abscesses and bacterial meningitis. During disseminated infection, the larvae themselves may enter the CNS with the development of gram-negative meningitis from enteric pathogens, and in some cases secondary abscesses.[51-54] 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.[55]

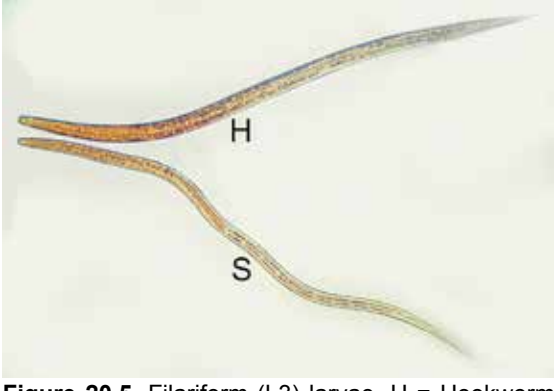


Figure 20.5. Filariform (L3) larvae. H = Hookworm and S = *Strongyloides*. Courtesy L. Ash and T. Orihel.

Figure 20.6. Buccal cavity (arrow) of the rhabditiform larva of *S. stercoralis*. Compare with Figure 19.8.

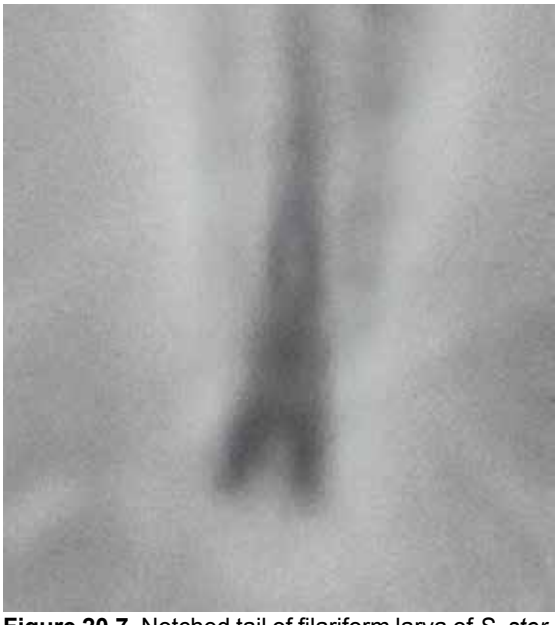


Figure 20.7. Notched tail of filariform larva of *S. stercoralis.* Hookworm L3 larva has a pointed tail.

Diagnosis (see Clinical Appendix)

Strongyloides infection should be considered in any patient with unexplained gastrointestinal (GI) symptoms, with or without eosinophilia, and an appropriate exposure history.[56] Identification of the larvae in stool samples is the definitive method of diagnosis. Because few organisms are intermittently 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, 57, 58] As few as 50 larvae are released per day by each adult *Strongyloides*. Compare this reproductive output with that of *Ascaris*, which produces more than 200,000 eggs per day. 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 for seeing the organisms on microscopic examination (Figs. 20.3, 20.6). 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 hookworm (Fig. 20.5). The rhabditiform larva of *S. stercoralis* has a short buccal cavity (Fig. 20.6), while the L3 larva has 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 the preparation under conditions that permit the heterogonic life cycle to take place (coproculture).[59, 60]

In patients with hyperinfection or disseminated infection, the yield of *Strongyloides* larvae detection may be increased by recovering them from duodenal fluid.[58] 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 different serological tests, including ELISA for IgG, are available, but their sensitivities and specificities can vary significantly.[61-63] Despite their limitations, serologic testing is

currently the diagnostic method of choice. Immunological approaches for detecting parasite-specific antibodies in serum by ELISA can achieve diagnostic sensitivity around 85% 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.[64]

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.[65-69] Albendazole is an alternative drug, but is less effective than ivermectin.[70]

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.[65, 66, 71-73] Treatment efficacy can be monitored based on patient response and repeated stool examinations.[74] Ivermectin treatment significantly reduces the burden of parasites but does not appear to completely rid the host of parasites, 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.[75] In some cases of severe infection veterinary parenteral preparations of ivermectin have been used successfully in the treatment of disseminated strongyloidiaisis.[76, 77] Intensive supportive therapy, including antimicrobial agents, is often required in patients with hyperinfection from *S. stercoralis*, as well as parenteral nutrition to compensate for extensive protein and lipid losses. *S. fuelleborni* infection is treated successfully with thiabendazole.[78]

Prevention and Control

Disease risk may be greatly reduced by wearing shoes in endemic areas.[79] 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 of making sure that these serious pathogens do not cause in-house epidemics, such as the one that occurred at Hoechst in Marburg, Germany in 1967.[80]

Custodial institutions can be a foci of infection, and consequently screening and treating of patients can prevent spread.[81] 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 who are infected and of childbearing age would reduce transmammary transmission of *S. fuelleborni*, although many of the available antihelmintic medications are relatively contraindicated during pregnancy. Currently strongyloidiasis is not targeted for mass drug administration, as are ascariasis, trichuriasis, hookworm, lymphatic filariasis, and onchocerciasis. Given the widespread use of albendazole and ivermectin for this purpose, it is conceivable that strongyloidiasis is also now being treated as a collateral parasitic infection, or possibly it could be added as a sixth helminthiasis in a newly designed global de-worming program.[82]

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21. *Trichinella spiralis*
(Railliet 1896)

Pronunciation: \trik-ə-ˈnel-ə\

Introduction

The genus *Trichinella* (\TRIK-in-el-ah\) has 12 recognized species and genotypes with different geographical distributions. They are all capable of infecting humans.[1-9] The identified *Trichinella* spp. include: *T. spiralis, T. britovi, T. pseudospiralis, T. papua, T. nativa, T. nelsoni, T. murrelli, T. zimbabwensis,* and *T. patagoniensis*.[1, 10-12] Members of the genus *Trichinella* are able to infect a broad spectrum of mammalian hosts, making them one of the world's most widely distributed group 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 lives as intracellular parasites. All species of *Trichinella* are transmitted by ingestion of raw or undercooked infected meats.

Trichinellosis is the name given to the diseases caused by the *Trichinella* spp. Currently, prevalence of trichinellosis is low within the United States, occurring mostly as scattered outbreaks, less often involving pork consumption and more often involving poorly cooked game, with the majority of human cases being due to *Trichinella spiralis* and *T. murrelli*.[9, 13-15] The domestic pig is the main reservoir host for *T. spiralis*. This species is significantly higher in prevalence 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.[16] *T. spiralis* infection in humans was reported from Korea for the first time in 2000.[17] In contrast, nematode infections in wildlife within the United States are now thought to be largely due to *T. murrelli*.[18]

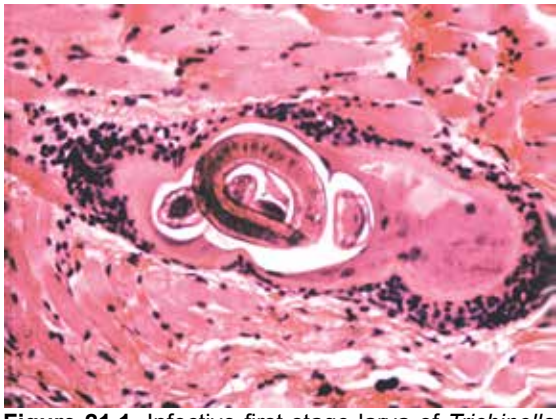


Figure 21.1. Infective first stage larva of *Trichinella spiralis* in its Nurse cell in muscle tissue. The worm measures 1mm x 36 μm.

An outbreak of *T. pseudospiralis* in Thailand was reported.[19] This species can also infect birds of prey. Foci have also been described in Sweden, the Slovak Republic and Tasmania (Australia).[20-22] *Trichinella paupae,* similar in biology to *T. pseudospiralis*, has been described in wild and domestic pigs in Papua New Guinea.[11]

Humans can also be infected with *T. nativa* and *T. britovi*.[23, 24] 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. There are numerous reports in the literature of infections with this parasite in fox, raccoon, dog, opossum, domestic and wild dogs, and cats.

T. nelsoni is restricted to mammals in Equatorial Africa, such as hyenas and the large predatory cats.[25] Occasionally people acquire infection with *T. nelsoni*.[26] 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 that has been associated with pansteatitis (generalized body fat inflammation) outbreaks in crocodile populations in many areas of Sub-Saharan Africa.[11, 27] *T. pseudo-*

spiralis has been isolated from the Tasmanian Devil, but not from humans living in that part of Australia.[22]

Historical Information

In 1821, the encysted larva was recognized in muscle but was not associated with disease in humans.[28] In 1835, a medical student at St. Bartholomew's Hospital in London by the name of James Paget discovered the worm in humans.[29] 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. The report of this discovery was published by Owen.[28] Both Rudolf Virchow and Friedrich Zenker, in 1859 and 1860, discovered the adult *Trichinella* worms.[30] Zenker was the first to recognize that eating raw pork could result in infection of humans with *Trichinella*.[31]

Life Cycle

There is both a domestic as well as a sylvatic cycle for *Trichinella*. The domestic cycle involves animals such as pigs and horses. The sylvatic cycle involves a very broad range of wild animals, including wild boar, bear, moose, cougars, crocodiles, foxes, birds and walruses.[32] In both cycles, infection is initiated by ingesting raw or, in the case of humans, 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.[33, 34] This enables the parasite to receive environmental cues and to then select an infection site within the small intestine.[35] The immature parasites penetrate the columnar epithelium at the base of the villus. They live within a row of these

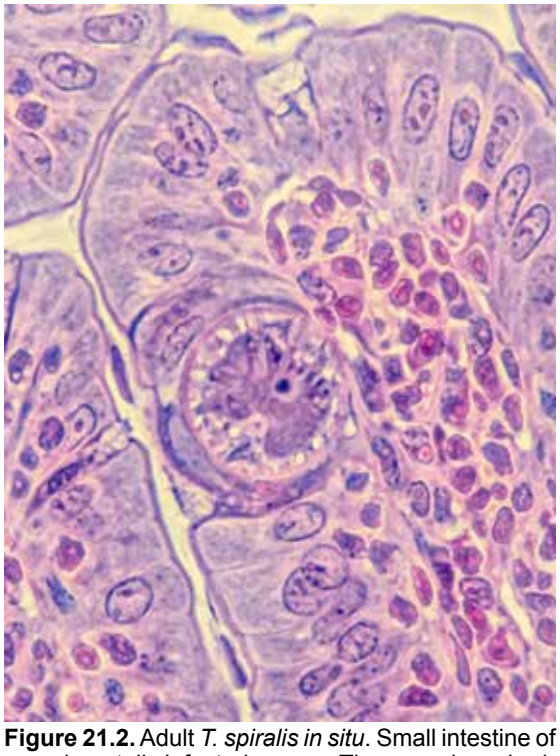
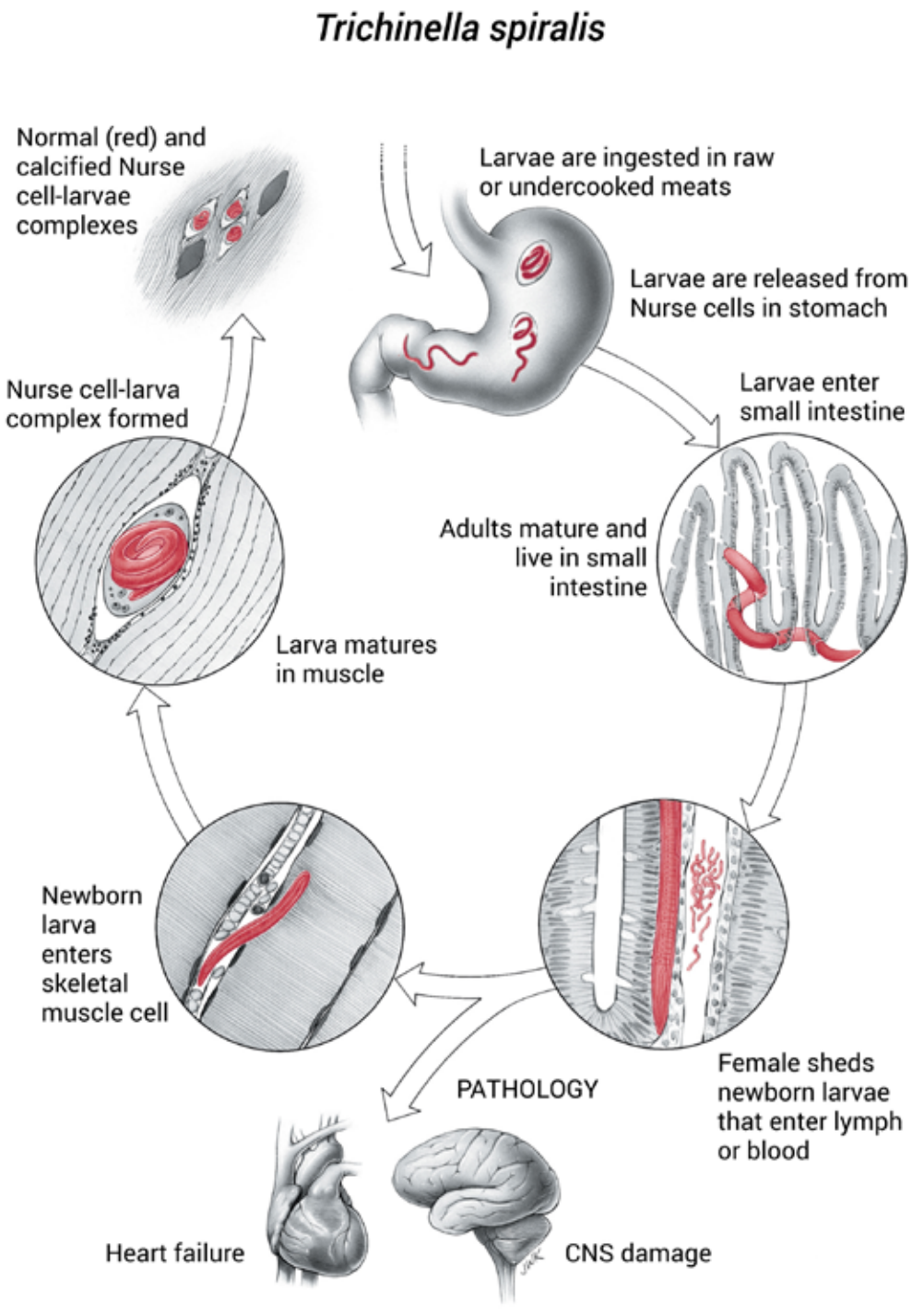


Figure 21.2. Adult *T. spiralis in situ*. Small intestine of experimentally infected mouse. The worm is embedded within the cytoplasm of the columnar cells.

Figure 21.3. Adult female *T. spiralis.* 3 mm x 36 μm. Note fully formed larvae in uterus.


Trichinella spiralis
Normal (red) and calcified Nurse cell-larvae complexes
Larvae are ingested in raw or undercooked meats
Larvae are released from Nurse cells in stomach
Larvae enter small intestine
Adults mature and live in small intestine
Nurse cell-larva complex formed
Larva matures in muscle
Newborn larva enters skeletal muscle cell
Female sheds newborn larvae that enter lymph or blood
PATHOLOGY
Heart failure
CNS damage

cells, and are considered intra-multi-cellular organisms (Figs. 21.2, 21.7).[36]

Larvae molt four times in rapid succession over a 30-hour period, 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, 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.[37] Eventually, acquired protective responses interfere with the overall process of embryogenesis and creates physiological conditions in the local area of infection which forces the adult parasites to egress and 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 only stage of the parasite that possesses a sword-like stylet,

Figure 21.4. Adult male *T. spiralis*. Note claspers on tail (lower end). 1.5 mm x 36 μm.

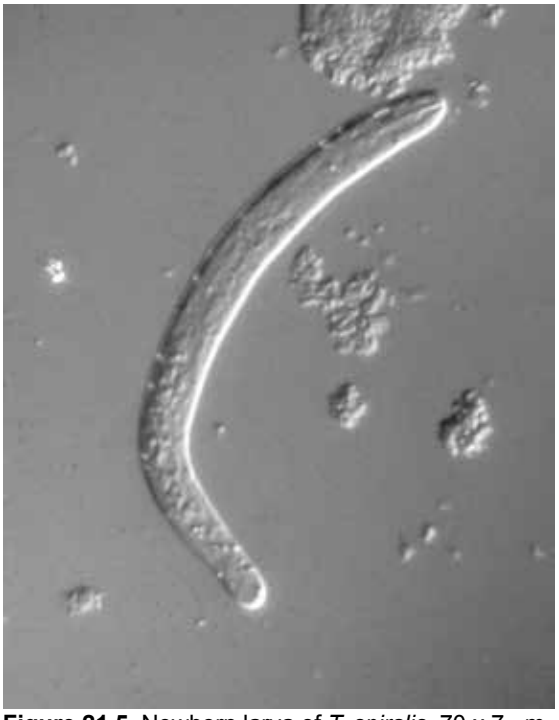


Figure 21.5. Newborn larva of *T. spiralis*. 70 x 7 μm.

located in its oral cavity. It uses it to create an entry hole in potential host cells. Larvae enter the lamina propria in this fashion, and penetrate into either the mesenteric lymphatics or the bloodstream. Most newborn 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 the result of invasion. Skeletal muscle cells are an exception.[38] Not only do the parasites remain inside them after invasion, 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.[39] Parasite and host cell develop in a coordinated fashion. *T. spiralis* is infective by the 14th day of infection, but

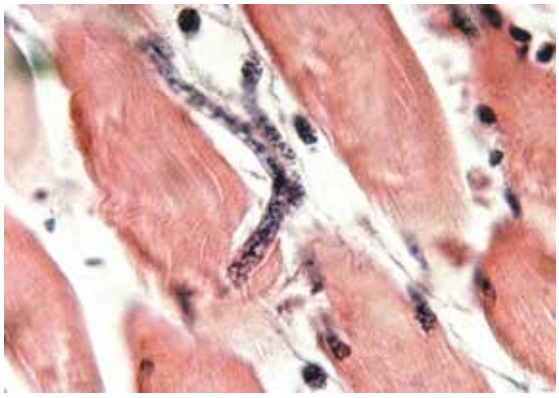


Figure 21.6. Newborn larva of *T. spiralis* entering muscle cell.

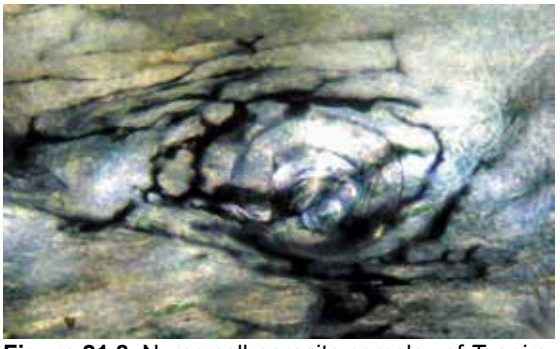


Figure 21.8. Nurse cell-parasite complex of *T. spiralis*, *in situ*. Infected mouse was injected with India ink to visualize circulatory rete.

the worm continues to grow in size through to day 20.[40] The significance of this precocious behavior has yet to be appreciated.

Parasites inside cells other than striated muscle cells fail to induce Nurse cells, and either reenter the general circulation or die. Nurse cell formation results in 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. The net result is that the host cell switches from an aerobic to an anaerobic metabolism.[41] Host cell nuclei enlarge and divide, amplifying the host's genome within the Nurse cell cytoplasm.[42, 43] 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. In order for the life cycle to continue, another mammal must eat infected host tissue. Omnivorous and carnivorous modes are common among wild mammals, and this helps to ensure the maintenance of *T. spiralis* and its relatives in their respective host species.

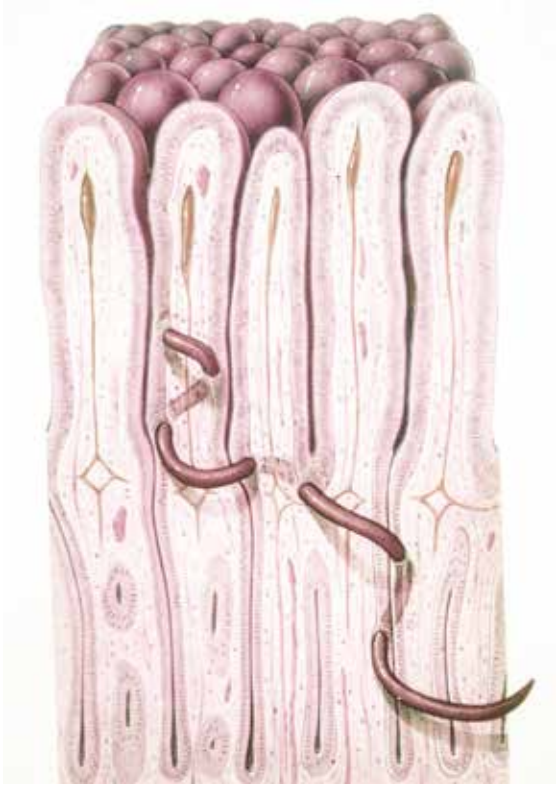


Figure 21.7. An adult female *T. spiralis,* depicted *in situ*. Drawing by J. Karapelou.

Cellular and Molecular Pathogenesis

The enteral (intestinal) phase includes larval stages L1–L4, the immature and reproductive adult stages. 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 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

Figure 21.9. Nurse cell-parasite complex. Phase interference. Photo by E. Gravé.

enough to result in malabsorption syndrome.

When larvae penetrate into the lymphatic circulation or bloodstream, a bacteremia due to 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.[44] Loss of wheat germ agglutinin receptors along the entire small intestine occurs.[45] The myenteric electric potential is interrupted during the enteral phase, resulting in a slow-down in gut motility.[45]

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. The result during heavy infection is a generalized edema. Proteinuria may ensue. Cardiomyopathies and CNS abnormalities are also common in those experiencing moderate to heavy infection.[1]

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. Induction of interleukin-4, and interleukin-13, as well as production of eosinophils and IgE antibodies appears to be essential for limiting production of newborn larvae and for the expulsion of adult worms.[46, 47] While host factors may lead to 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, appears to be essential for maturation of the Nurse-cell complex.[48] TNF-induced NO production is, however, not one of the effector mechanisms, since knockout mice unable to produce NO expelled their parasites in a normal fashion in the absence of local gut damage.[49] In NO^+ mice, 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

The presentation of the disease varies over time, and, as a result, resembles a wide variety of clinical conditions (Fig. 21.10).[1, 50] Trichinellosis is often misdiagnosed for that reason. The severity of disease is parasitemia-dependent, making the diagnosis based solely on symptoms difficult, at best. In severe cases, death may ensue. There are signs and symptoms that should alert the physician to include trichinellosis into the differential diagnosis.

While light infection with *Trichinella* can pass unnoticed with minimal or no identifiable symptoms, heavy infection with a large number of viable larvae presents as a distinct

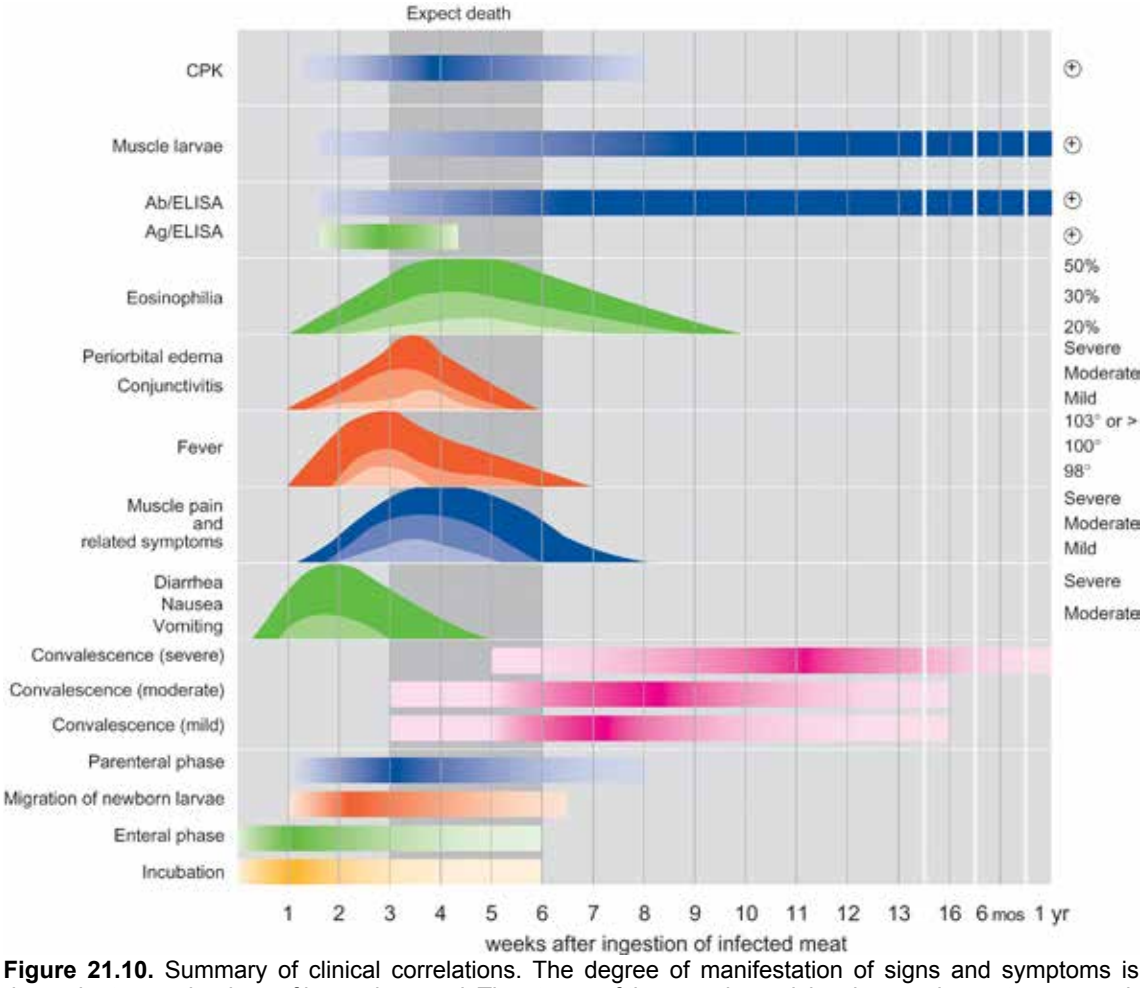


Figure 21.10. Summary of clinical correlations. The degree of manifestation of signs and symptoms is dependent upon the dose of larvae ingested. The stages of the parasite and the signs and symptoms associated with them are shown in the same colors.

two-stage syndrome.[7] The first few days of the infection are characterized by the GI stage with gastroenteritis associated with secretory diarrhea, abdominal pain, and vomiting.[51]

This phase is transitory and abates within 10 days after ingestion of infected tissue. A history of eating raw or undercooked meats helps to rule in this parasitic infection. Others who also ate the same meats and are suffering similarly reinforce the suspicion of trichinellosis. Many clinicians 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.[52] Muscle tenderness can be readily detected. Laboratory studies reveal a moderately elevated white blood cell count (12,000–15,000 cells/mm^3), and a circulating eosinophilia ranging from 5% to as high as 50% (usual absolute eosinophil range 600–7,500 cells/mm^3).[53, 54]

Larvae penetrating tissues other than skeletal muscle may give rise to serious sequelae. In many cases of moderate to severe infection, cardiovascular involvement may lead to myocarditis, but this aspect of the infec-

tion has been overrated as a clinical feature typical of most infections with this parasite, since most instances encountered by the clinician are of the mild variety.[55] Electrocardiographic changes can occur during this phase, even in the absence of symptoms. Parasite invasion of the diaphragm and the accessory muscles of respiration may result in dyspnea.[56] Neurotrichinellosis occurs in association with invasion of the CNS.[57] In up to 25% of patients with heavy infection, meningitis and encephalitis can develop with edema, infarcts, and hemorrhage in fatal cases.[58, 59] The convalescent phase follows the acute phase, during which time many, but not all, Nurse-cell-parasite complexes are destroyed.

Two clinical presentations have been described for *T. nativa* infections resulting from the ingestion of infected polar bear or walrus meat: a classic myopathic form, and a second form that presents as a persistent diarrheal illness. The second form is thought to represent a secondary infection in previously sensitized individuals.[60] It is apparent that not only the amount of ingested larvae but also the species of *Trichinella* and the status of the infected host impact the clinical presentation.[61]

Diagnosis (see Clinical Appendix)

Definitive diagnosis depends upon finding the Nurse cell-parasite complex in muscle biopsy by microscopic examination (Fig 21.1), or detection of *Trichinella*-specific DNA by PCR.[62] PCR is sensitive and specific for detecting small numbers of larvae in muscle tissue, but is rarely used in the clinical setting.[52] Real-time PCR has been developed and is used in surveillance studies of wildlife *Trichinella* infection.[63]

Muscle biopsy can be negative, even in the heaviest of infections, due to sampling errors and 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.[1] In addition, the larvae may be at an early stage of their development, making them inconspicuous, even to experienced pathologists. Serological tests are available with confirmatory Western blot testing.[64] 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 armed with this information and an exposure history, 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.[65-67]

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 a combination of antihelmintic therapy and corticosteroids.[52] Since larvae may still be burrowing through the GI tract when patients present with symptoms, treatment with albendazole or mebendazole may be beneficial. Treatment with mebendazole early in the course of infection has been associated with decreased muscle pain and muscle inflammation.[68] Once muscle invasion has occurred, treatment with these agents will not succeed in destroying encysted larvae.[69] Anti-inflammatory corticosteroids, particularly prednisolone, are recommended

if the diagnosis is secure.[1, 49] The myopathic phase is treated in conjunction 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 10 years, outbreaks of trichinellosis in the United States have been rare and sporadic in nature.[13-15, 70] Most have been associated with the ingestion of raw or undercooked meats from game animals, and not from commercial sources.[13-15] This represents a shift in the epidemiology of outbreaks compared to 20–30 years ago, when commercial sources of infected pork were much more common than today. Pigs raised on individual farms, as compared with commercial farm operations, are more likely 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 10 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 alike.[70]

As already mentioned, top carnivores such as bear, fox, cougar, and the like often become infected. Hunters, and those sharing their kill are best warned to cook all meat thoroughly. Herbivores can harbor the infection as well, since most plant eaters occasionally ingest meat when the opportunity arises. Epidemics due to eating raw horsemeat have been reported from France, Italy, and Poland.[71]

Meat inspection is nonexistent in the United States with respect to *Trichinella*. 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. If they are consistently negative, then a *Trichinella*-free designation is applied to that supply of meat. Rare outbreaks occur, despite this rigorous system of inspection.

Trichinellosis due to *T. spiralis* derived from pork can be prevented by either cooking meat thoroughly at 58.5 °C for 10 minutes or by 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.[72] *T. nativa* was found to survive for up to 34 months in frozen muscles of grizzly bear and up to 5 years frozen at -18 °C in the muscles of carnivores.[5] It appears that freeze tolerance of encapsulated *Trichinella* muscle larva is largely determined by *Trichinella* species, but may also be dependent on aspects of host tissue.[72-74] 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.

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22. Lymphatic Filariae

Wuchereria bancrofti
(Cobbold 1877)

Pronunciation: \wůk-ə-ˈrir-ē-ə\ \ ban-krȯft-ī \

Brugia malayi
(Brug 1927)

Pronunciation: \brü- jē-ə\\ ma-lē-ī\

Brugia timori
(Partono 1977)

Pronunciation: \brü- jē-ə\\ tē-ˌmȯr\

Introduction

There are three species of vector-born nematodes that 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] More than 100 million people in Southeast Asia, Africa, and the Americas are infected with some form of filariasis, with more than 90% due to *W. bancrofti* infection.[3, 4] Of these, approximately 40 million suffer from clinical disease. Only about 10–20 million people are infected with *B. malayi*, while *B. timori* is a minor filarial parasite largely restricted to the islands of Timor and Flores in southeastern Indonesia.[5] 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.

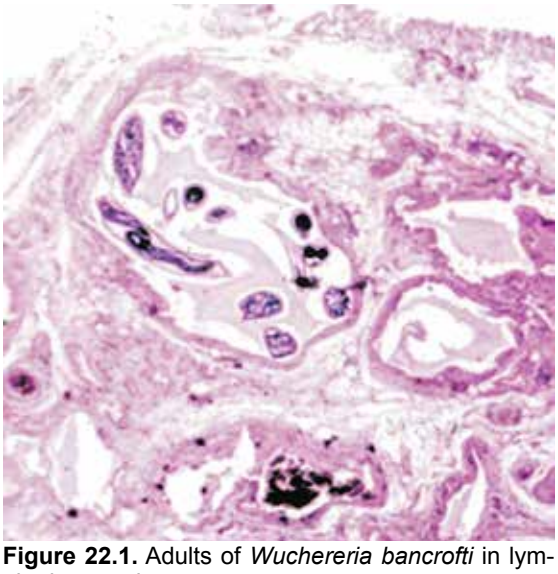
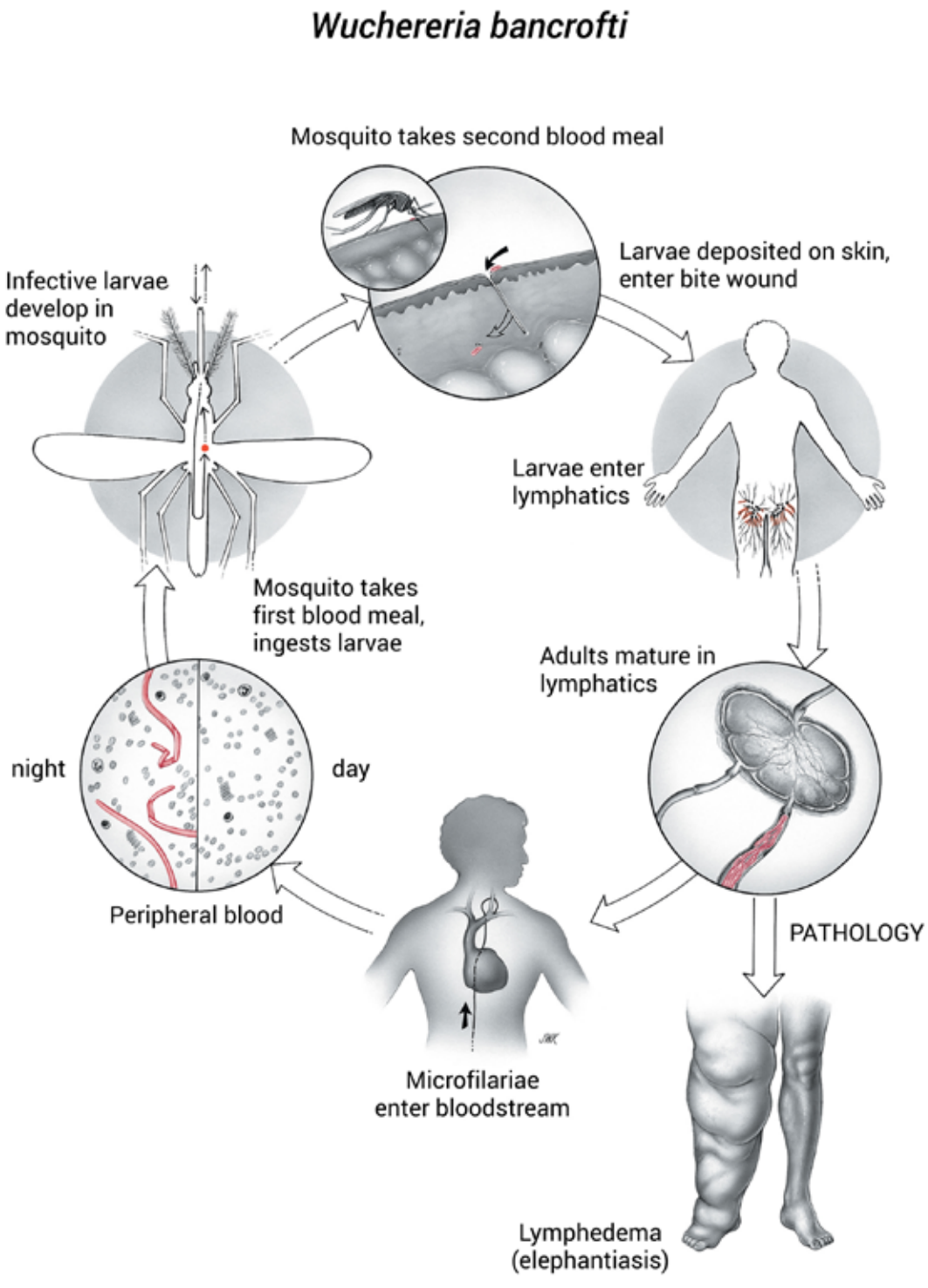


Figure 22.1. Adults of *Wuchereria bancrofti* in lymphatic vessels.

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. With the exception of 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*, on the other hand, is a zoonosis, with both feline and monkey reservoirs. *Mansonia* spp. serve as the major mosquito vector, although anophelines are also sometimes involved in transmission. *B. malayi* infections occur in India, Malaysia, and other parts of Southeast Asia. There are other minor members of the genus *Brugia* that 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.[6, 7]

A major global effort was initiated to eliminate LF by the year 2020, based on the successes of reducing the age-adjusted prevalence but this has not been achieved.[8-10] The term ‘elimination’ refers to the reduction of disease incidence to zero or close to zero in


Wuchereria bancrofti
Mosquito takes second blood meal
Larvae deposited on skin, enter bite wound
Infective larvae develop in mosquito
Larvae enter lymphatics
Mosquito takes first blood meal, ingests larvae
Adults mature in lymphatics
night
day
Peripheral blood
PATHOLOGY
Microfilariae enter bloodstream
Lymphedema (elephantiasis)

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.[11] The strategy for LF elimination relies on interrupting mosquito transmission by mass administration of combination therapy in endemic regions in order 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.[12] In 1866, Otto Wucherer described the microfilariae in urine in Brazil.[13] In 1872, a Scottish physician working in Calcutta, Timothy Lewis, confirmed the presence of the microfilariae in urine as well as blood and recognized the connection with the severe lymphedema and deformities known as elephantiasis.[14, 15] 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.[15, 16] Lewis described the adult worm in India that same year.[17] In 1878, Patrick Manson completed the description of the life cycle while working in Amoy (now called Xiamen) along the Chinese coast in Fujian Province.[18] 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 came to accept the concept that larvae were transmitted by the bite of mosquitoes. Filariasis may, in fact, be a water-borne disease under some circumstances, since experimental infections can be induced by the oral route.[19]

One of the most important developments in the history of LF control was the discovery by Francis Hawking (father of Stephen 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).[20, 21] During the 1970s and 1980s, Chinese parasitologists scaled up this approach to the national level. This was achieved primarily through fortification of regional salt supplies with DEC. The LF life cycle was discovered in China and LF was first eliminated there as well. The accomplishments of the Chinese provide proof-of-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 *Wuchereria* females are 3–10 cm in length while the adult *Wuchereria* males are about half this length. Adult *Brugia* females are smaller, measuring 4–5.5 cm in length,

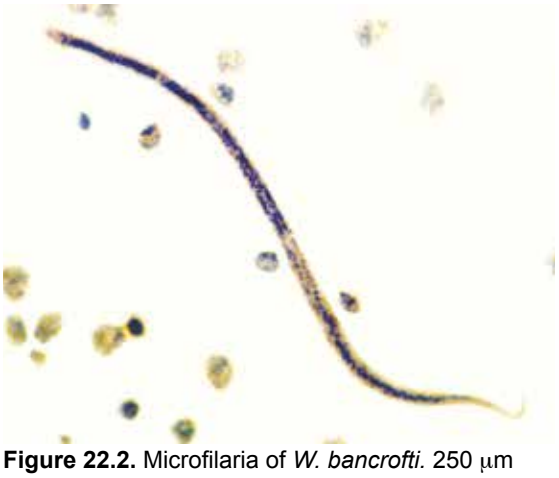


Figure 22.2. Microfilaria of *W. bancrofti.* 250 μm

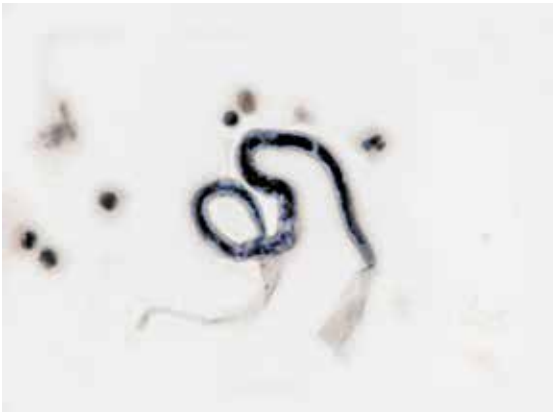


Figure 22.3. Microfilaria of *Brugia malayi*. 220 μm

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 μm by 10 μm 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. They are typically present in large numbers in the peripheral blood only at night (22:00–06:00) in most endemic areas of the world. During the day, the microfilariae aggregate in the capillaries of the lungs when activity of the host is increased (i.e., during strenuous exercise). Nocturnal periodicity could be a result of the microfilaria's penchant for low oxygen tension, at which time they are found in the peripheral blood stream, or it may reflect subtle pH changes in the pulmonary venous circulation during sleep.[22] Experiments in which sleep habits of infected volunteers were reversed also reversed the periodicity of microfilariae. The diurnal periodicity pattern 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, Culex pipiens pipiens, Anopheles gambiae,* and *A. polynesiensis. Aedes aegypti*, the yellow fever mosquito, can also transmit the infection in some of the 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 become infective after 10–20 days of growth and development in the insect muscle tissue.

Infective L3 larvae locate to the 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 come to rest near the draining lymph nodes of each of those vessels. The worms slowly develop into mature adults, taking about 1 year. Soon after copulation they begin shedding microfilariae. The longevity of adults, measured by the continuous production of microfilariae, is estimated at 5–8 years. Infections lasting 40 years have been reported.[23]

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 animals, and transmitted through the bite of *Mansonia* spp. mosquitoes.[6]

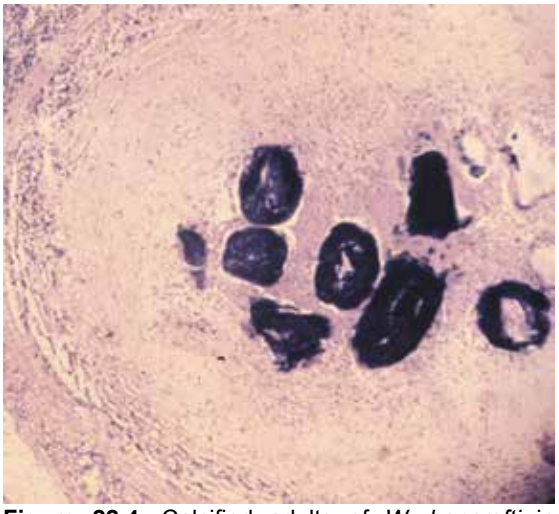


Figure 22.4. Calcified adults of *W. bancrofti* in blocked lymphatic vessel.

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.[24, 25] The processes associated with lymphangitis and elephantiasis can take years to develop, and are not commonly seen in children. Exactly 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.[26] Adult worms can also influence host immune responses through the release of small RNA-containing exosome-like vesicles that influence host immune-cell gene expression.[27]

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 lumen of the vessel 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 region of the body. In addition, hypertrophy of smooth muscle tissue occurs in the area immediately surrounding the site of involvement. Individuals can develop complications, such as the development of new hydroceles when treated with agents that kill adult filarial worms.[28] The process of lymphatic blockage is a protracted one and results from repeated infections. Consequently, individuals 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 with evidence of microfilaremia, whereas other individual's progress to advanced clinical disease comprised of lymphangitis and elephantiasis. Frequently, patients with advanced clinical disease do not have evidence of circulating microfilariae, while patients with elevated levels of circulating microfilariae are often asymptomatic.[29] 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.[24, 30, 31] Growing evidence suggests that host genetic variability accounts for the different range of clinical manifestations seen among patients with similar exposures.[32, 33]

There is a complex relationship between the host and the parasite, and there is often a complex pathologic sequence of events lead-

ing 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, as well as being significant complications for patients with full-blown elephantiasis. Adult *W. bancrofti* worms harbor bacterial symbionts of the genus *Wolbachia*. Adult *W. bancrofti* depend on these symbionts for their survival, and antibiotics that target these symbionts exhibit an antihelmintic effect. Further, *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.[34-37]

Clinical Disease

There is a spectrum of clinical manifestations resulting from *W. bancrofti* or *B. malayi* infections, ranging from asymptomatic infection to advanced elephantiasis.

Asymptomatic Infection (Lymphatic Dilatation)

The majority of residents living in an endemic area do not manifest strong inflammatory responses to their filarial parasite load. They are noted to be 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.[38] 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.[39] In some cases, the dilated vessels rupture to produce chyluria and chylocele.

Acute Lymphadenitis and Filarial Fevers

Death of the adult worm causes the next step in 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 adolescent years, and is manifested with fevers and painful swellings over the lymph nodes.[40] This typically occurs in the inguinal area. Episodes of painful swellings can last up to a week and commonly recur. Acute filarial lymphadenitis may be exacerbated by secondary bacterial infections.

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.

Some individuals who have travelled 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 war in the Pacific theatre.[41]

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 and exhibits some degree of pitting, though it is rather firm. As the inflammatory reaction continues, the area becomes firmer still, 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 are 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.[42] 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).[42]

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, 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, can be used for daytime testing, and can give a quantitative result correlating with adult worm burden.[43-45] These are available in the form of ELISA test or an immunochromatographic card.[46] The card-based assay, which recognizes an adult worm's 200 kDa antigen, has a sensitivity of 96–100% and a specificity approaching 100%.[47] The ELISA also has a sensitivity approaching 100% in microfilare-

mic 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, are currently not routinely used in clinical practice.[48]

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 the diagnosis of this disease. Note, they detect exposure rather than confirm current infection.[49-51]

Increasingly, ultrasound has provided an important noninvasive modality for monitoring the efficacy of antifilarial drugs.[34] 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.[52] 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.[53] It is critical that, prior to treatment, co-infection with *Loa loa* with a high *Loa loa* microfilarial load is ruled out, due to the risk of severe adverse events if treatment is given to such patients.[54] For treatment of mono-infected patients, DEC has both 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.[34, 55] 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.[56-58]

In men, the efficacy of treatment can be monitored by serial ultrasound examinations (see above), and by serial blood sampling.[34] Since DEC is only partially effective against the adult worm, repeat treatments are often required. This is often done every 6–12 months.[34] 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.[34] DEC is associated with fever (probably resulting from disintegration of a few of the adult worms), occasional nausea and vomiting, and fleeting skin rashes. Ivermectin, a drug effective for therapy of onchocerciasis, also kills microfilariae of *W. bancrofti*, but it appears to have no macrofilaricidal properties. Ivermectin and albendazole, a drug that also does not kill adult filarial worms, have been used in mass drug campaigns, reducing microfilarial blood levels and decreasing transmission.[59]

Aside from the use of antihelmintic 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.[34] Treatment of secondary bacterial infec-

tions has been identified as a critical treatment modality for lymphedema and elephantiasis. Hydrocele drainage without corrective surgery, while it does provide relief, is often associated with reaccumulation of fluid.[60] For certain affected areas, (e.g., the scrotum) corrective surgical interventions may be required.[61] Surgical techniques have been developed that can, in most cases, alleviate hydroceles and greatly improve the morbidity associated with filarial infections.[62]

Prevention and Control

Patent microfilaremia is first detected in children 5–10 years old who live in endemic regions.[6] Transplacental immunity and breastfeeding may limit the intensity of infection in younger individuals. The prevalence of microscopically confirmed infection gradually increases up to the age of 30–40 years.

The frequency of exposure to L3 by vectors is the most important determinant in the community prevalence of filariasis.[63] Prevention depends upon control of mosquito vectors, which has had limited success because mosquitoes develop resistance to insecticides. Insecticide-treated bed nets have been effective in reducing transmission in areas where anopheline mosquitos transmit the disease.[64] Urbanization of vast areas of tropical Asia has resulted in a concomitant rise in the prevalence of both *W. bancrofti* and *B. malayi* varieties of filariasis carried 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. To interrupt transmission, it is essential to reduce the levels of microfilariae in the blood for a sustainable period. This is achieved by administering a yearly, single-dose, 2-drug regimen.[65] For most countries, the recommended drugs are DEC (6 mg/kg) and albendazole (400 mg).[65, 66] The goal of this approach is to provide annual treatment with this drug combination.

In many parts of Sub-Saharan Africa (and Yemen as well) 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 ivermectin or albendazole.[64, 66] A period of 5 years of annual treatments is currently recommended. To date, 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.

To alleviate suffering and decrease the disability caused by LF, the major strategy 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.

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23. *Onchocerca volvulus*
(Leuckart 1893)

Pronunciation: \äŋ-kə-ˈsər-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 a variety of anatomical sites contiguous with that tissue. There are no reservoir hosts for *O. volvulus*, the usual species responsible for human disease.[1] The black fly, *Simulium* (\ seh-MYOO-LEE-um\,\si-ˈmyü-lē-əm\) spp., is the vector of *O. volvulus*. This filarial parasite occurs mostly in West and Central Africa, except for foci in Yemen in the Middle East, while in the Americas this disease is rapidly being eliminated with the exception of foci among indigenous populations living on the Brazil-Venezuela border.

Onchocerciasis used to be the major cause of blindness throughout Sub-Saharan Africa, often affecting more than 50% of the inhabitants of towns and villages in endemic areas.[2] River blindness is now the second-leading infectious cause of blindness in the world with approximately half a million people being blind due to *O. volvulus*.[3] The disease also causes a 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.[4]

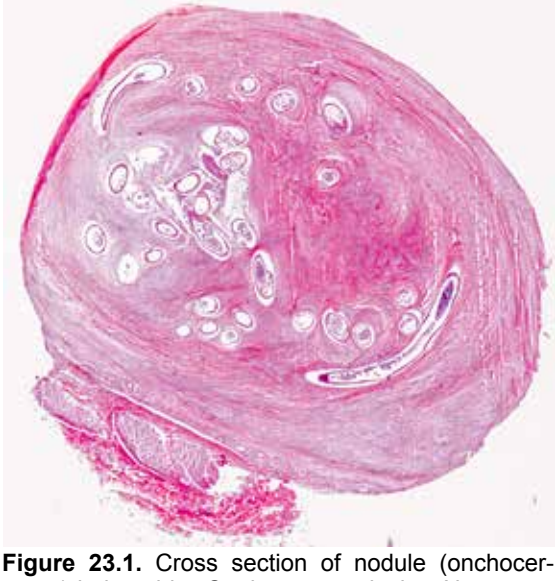
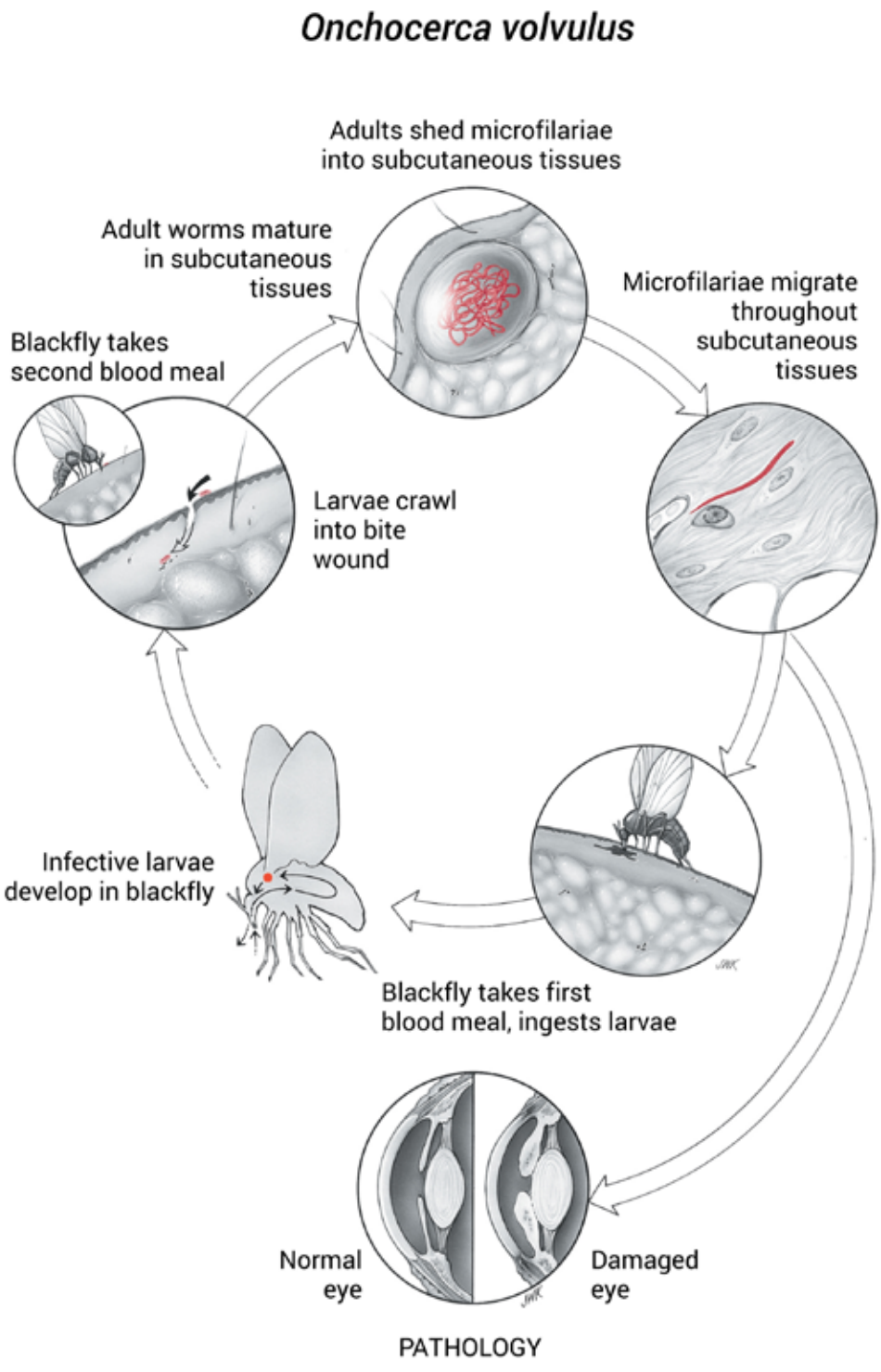


Figure 23.1. Cross section of nodule (onchocercoma) induced by *Onchocerca volvulus*. Numerous sections of adult worms are seen. 2.5 cm diameter.

Current estimates indicate that approximately 17 million people remain infected worldwide, with 99% or more living in Sub-Saharan Africa. Since 1995, an African initiative, the African Programme for Onchocerciasis Control (APOC), a partnership under the leadership of the World Bank, the World Health Organization, the United Nations Development Programme, and the Food and Agriculture Organization of the United Nations, has coordinated the annual mass treatment of ivermectin in 19 countries and has had a highly cost-effective impact on controlling this disease (see book dedication).[5] This organization has built on the previous successes of the Onchocerciasis Control Program (OCP), and there is optimism that onchocerciasis might be eliminated in the coming decades. APOC aims to extend its reach to all of the remaining 19 endemic countries in Central and East Africa (Angola, Burundi, Cameroon, Central African Republic, Chad, Democratic Republic of Congo, Equatorial Guinea, Ethiopia, Gabon, Kenya, Liberia, Malawi, Mozambique, Nigeria, Rwanda, Sudan, Tanzania and Uganda).[5, 6] Similarly the Onchocerciasis Elimination Program for the Americas (OEPA) is working to eliminate river blindness in Latin America as well as South America.[7] Currently, thanks in large part to the efforts of the OEPA, active transmission is limited to two foci in Venezuela and Brazil.[7] Elimination of this disease is so close to being achieved that the World Health Organization


Onchocerca volvulus
Adults shed microfilariae into subcutaneous tissues
Microfilariae migrate throughout subcutaneous tissues
Blackfly takes first blood meal, ingests larvae
Infective larvae develop in blackfly
Larvae crawl into bite wound
Blackfly takes second blood meal
Adult worms mature in subcutaneous tissues
Normal eye
Damaged eye
PATHOLOGY

has recently issued guidelines for when to stop mass treatment programs and for verifying the elimination of the parasite from a given geographic region.[8]

Historical Information

In 1874, John O'Neill, an Irish naval surgeon, discovered microfilaria in skin snips of individuals in Ghana.[9] 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.[10] 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.[11] He dissected the nodule and found that it contained the adult worms. Later Robles described the anatomy of the worm, the pathology of the disease, and the epidemiology of the infection. Moreover, he suspected that the black fly was the vector, which was later proved by Donald Blacklock in 1927.[12]

Life Cycle

Adult females measure 20–80 cm by 0.3 cm, while the male measures 3–5 cm in length. Both sexes lie entwined about each other, locating to subcutaneous fibrous nodules, onchocercomas (Fig. 23.1), which vary in size depending on the number of adult worms in them. The male worms are able to migrate between nodules to fertilize different females, likely in response to chemoattractants released by female worms.[13] Some nodules are so small that they cannot be palpated.[14] Microfilariae are produced within the nodules, and leave 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, as males do not feed on blood. The black fly injects anticoagulants creating a blood meal from which it ingests blood and microfilariae.[15] 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 cavity of 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.[16] After completing their development, they mate.

There is a pre-patent period of 10–12 months after initial infection before the female worms start to produce microfilariae. Adults produce hundreds to thousands of microfilariae during their life span of 8–10 years (about 700 microfilariae per day). Growth and molting of worms in the subcutaneous tissues induces formation of the fibrous nodules and also elicits an angiogenic response, resulting in the production of a network of vessels, the function of which is presumably to supply nutri-

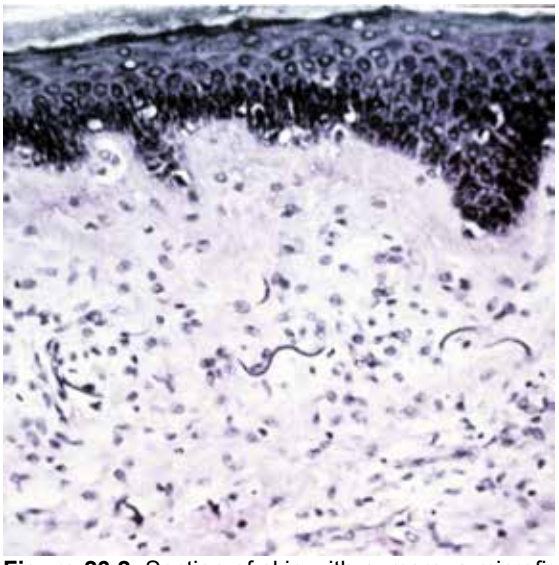


Figure 23.2. Section of skin with numerous microfilariae of *O. volvulus*.

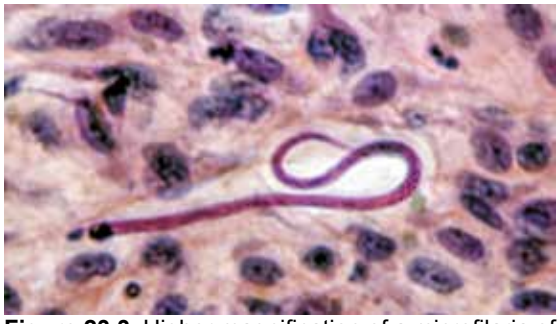


Figure 23.3. Higher magnification of a microfilaria of *O. volvulus* in skin. 310 μm x 7 μm.

ents to the parasites and carry away metabolic wastes.[17, 18] A similar angiogenic response is induced by the Nurse-cell-parasite complex of *Trichinella spiralis*.[19]

O. volvulus parasites contain bacterial symbionts of the genus *Wolbachia,* as do other filaria such *Wuchereria bancrofti*. These are rickettsia-like organisms that are found in the body wall, in oocytes, and in all embryonic stages, including microfilariae.[20] The *Wolbachia* symbionts are believed to be essential for nematode fertility and are transmitted transovarially to the next worm generation, in a manner similar to mitochondria.[21] *Wolbachia* also contain endotoxin-like products that are proinflammatory. This has led to the hypothesis that the bacterial symbionts contribute significantly to the skin and eye pathology of *O. volvulus*-infected patients.[20, 21]

Cellular and Molecular Pathogenesis

Onchocerca larvae migrate through the tissues with the aid of macromolecules that promote tissue degradation, angiogenesis, and plasmin-mediated proteolysis.[16-18, 22] *O. volvulus* has impressive immunomodulatory properties, with the capacity to 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.[23-25] Down regulation of the host immune response is thought to be induced by excretory-secretory products of *O. volvulus,* such as secretory omega-class glutathione transferase 3 (OvGST).[26]

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* endosymbiont bacteria-derived antigens upon the death of the filariae are thought to play an essential role in triggering the destruction caused by the host's innate immune cells.[27, 28] 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 as a consequence of cell-mediated immunity to parasite antigens. Individuals with the most vigorous cell-mediated immune responses develop the most severe manifestations.[2, 22, 29] The magnitude of the immunopathologic response significantly influences the severity of clinical onchodermatitis.[29, 30] Host mast cells play an important role in this phenomenon.[30]

The major ocular lesions occur in the cornea to produce a keratitis.[31] 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 with a heavy reliance on host interleukin-4, as well as neutrophil recruitment to the corneal stroma.[31, 32] 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.[33] In the skin, this leads to pruritus and angioedema.

The pathogenesis of onchocerciasis is dependent on the number of worms infecting an individual as well as certain host factors. Polymorphisms in the *FcyRIIa* gene that impact binding affinities of immunoglobulin subclasses have been correlated with clinical outcomes.[29] Genome-wide association studies have detected genetic variants associated with disease.[34] 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.[35]

Subcutaneous nodules, the other hallmark of clinical onchocerciasis, vary in size from barely discernable to approximately 5 cm in diameter. Nodules develop over an 18-month period depending on the number of adult worms in each. The number of nodules also varies, from an occasional one to several hundred, occupying large areas of subcutaneous tissue. In the latter instance, black flies biting such individuals may actually expire due to the overwhelming nature of the infection in their flight wing muscles. Those areas in which 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 of the body. This difference is related to the biting habits of the vector insects, and the styles of clothing worn by the inhabitants of each endemic area.

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).[36-38] 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.[39] 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.[40, 41] There have been rare descriptions of this occurring outside these geographical confines including cases reported in the New World.[42, 43]

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.[36, 37] Hanging groin can be associated with intense pruritus and the development of hernias.[44, 45] 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.[4, 46, 47] Occasionally, the pruritus of onchodermatitis is intense and disabling. It is alleged that occasional suicides result from the extreme discomfort associated with it.[2] A reported manifestation in infected Central American children involved facial lesions that were reddish in color, described as *erysipelas de la costa*.[48]

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.[2]

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.[49] 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 be the result of damage due to immune cells that are triggered by antigens from microfilaria and the *Wolbachia* 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.[49] Onchocercal blindness peaks in those between 30–40 years of age; when individuals are most responsible for taking care of their families. There is little evidence suggesting that sclerosing keratitis can be reversed sufficiently to restore vision once it has developed.[50]

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, although 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, especially in Uganda and in neighboring countries.[51] Although nodding syndrome has not been directly linked to onchocerciasis an epidemiological association has been noted.[52] Children with nodding syndrome exhibit periods of seizures and unresponsiveness comprised of multiple head nods and lack of responsiveness, as well as long-term mental disabilities. The etiology of nodding syndrome is under investigation.

Diagnosis (see Clinical Appendix)

Because of its highly focal distribution, a travel history or history of living in an endemic area is critical in order 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 mm^2 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 levels of microfilaria. 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 microfilar-

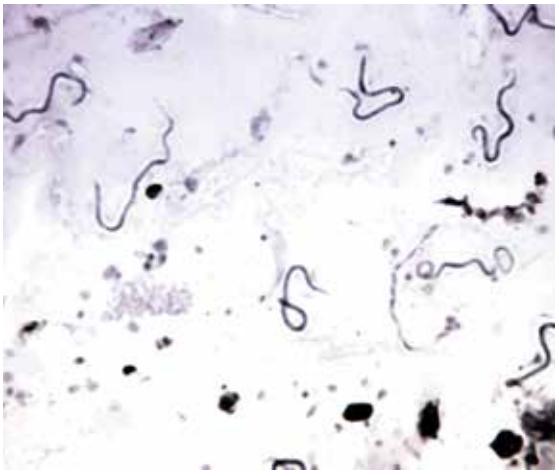


Figure 23.4. Impression smear of a skin snip from a patient heavily infected with *O. volvulus*. Microfilariae were visualized with Giemsa stain.

iae (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.[53] Histologic sections of a subcutaneous 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.[54, 55]

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.[56] 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.[57]

Ultrasound has been used to visualize adult worms in nodules as well as to monitor their viability following the initiation of therapy.[58]

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.[59] A single oral dose of 150 mcg/kg administered every 6 months can slow or reverse the progression of both ocular and cutaneous diseases.[60] 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 entire 10–17-year life span of the adult worms. In some patients more frequent interval dosing is required in order 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.

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.[61] 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* endosym-

bionts 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.[62] Prolonged administration of doxycycline (200 mg/day for 4–6 weeks) was shown to interrupt *O. volvulus* embryogenesis.[20]

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 they have a fairly long flight range. Thus, onchocerciasis can be found several miles from the nearest endemic breeding site. Because much of the coffee of the world is grown on mountainous hillsides, the prevalence of onchocerciasis among workers on coffee plantations is high. The OCP was launched in 1974, with a primary emphasis on reducing black fly larval vector populations with DDT and other insecticides.[63] With the increasing availability of ivermectin, the OCP has increasingly focused on control using this drug as an agent of mass chemotherapy.[64] Community-wide chemotherapy can interrupt transmission of onchocerciasis.[65, 66]

In Africa, efforts to control onchocerciasis are currently being conducted by the APOC.[6] Critical to the success of APOC is the Merck 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 an historic announcement that his company would donate Mectizan® to anyone who needed it, for as long as it was needed.[6, 64] The program works closely with the Carter Center and the Task Force for Child Survival and Development, both Atlanta-based non-governmental organizations, for this purpose. By 2004, Merck had already donated more than 300 million treatments worth over $450 million.[6]

APOC works with the organizations previously involved with the OCP, as well as Merck, the governments of 19 developing countries, 27 donor countries, at least 30 non-governmental organizations, and more than 80,000 rural Africa communities. This is done by coordinating with the ministries and non-governmental organizations to deliver Mectizan along with existing national health systems of the participating African countries. To accomplish its mission, APOC has implemented a novel system of community-directed treatment programs. It is likely that the sight of more than 500,000 people has been saved so far. In addition, the community-based health systems created by APOC are expected to provide a framework for additional pro-poor health interventions 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 greatly reduce the transmission of this parasite throughout the world and currently there are only limited areas of active transmission remaining.[5-7] Working with the Carter Center the number of people at risk for onchocerciasis continues to decline. APOC reached the end of its lifespan and there are now a new generation of control programs integrated with intestinal helminth infections, schistosomiasis, lymphatic filariasis and trachoma.

As a complementary approach to onchocerciasis control, there have been some efforts to develop recombinant vaccines.[64, 67] This program, the Onchocerciasis Vaccine for Africa, includes the development of a vaccine containing two antigens, Ov-103 and Ov-RAL-2.[68-71] 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.[70, 72]

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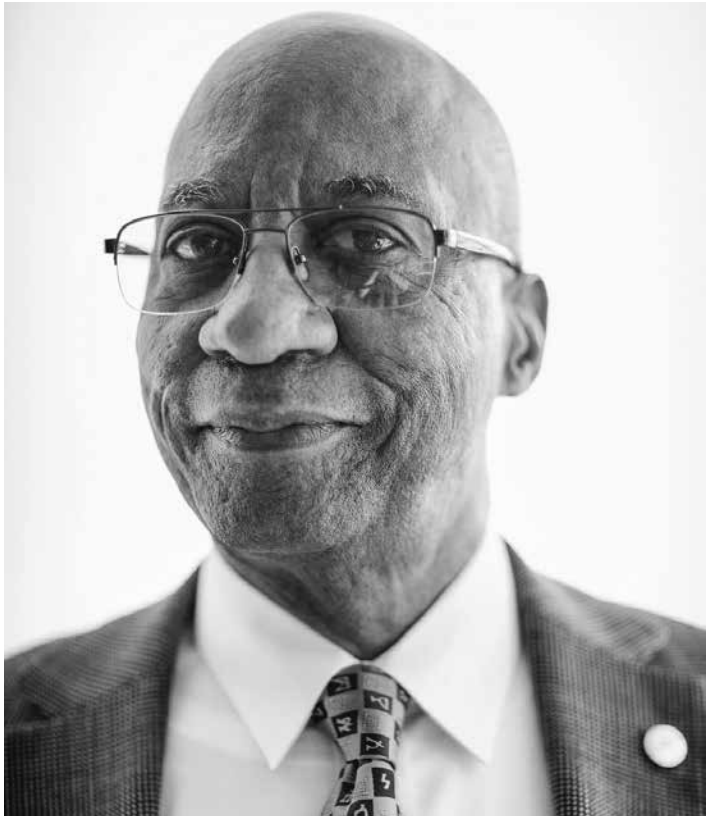


Donald Hopkins, M.D., M.P.H. (born 1941)

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 acquired in Central and West Africa, where it infects millions of individuals in the areas endemic for this pathogen.[1] In some hyperendemic regions, prevalence may be as high as 40%.[1, 2] Loiasis is an emerging infection in areas where the establishment of rubber plantations has altered the rainforest ecology.[3] *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.[4] 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 for onchocerciasis. The adult worm lives in subcutaneous tissues. Its main vectors are dipteran flies of the genus *Chrysops* ((\KRIS-ops\, \kris-ˌäps\), known as the deer fly.

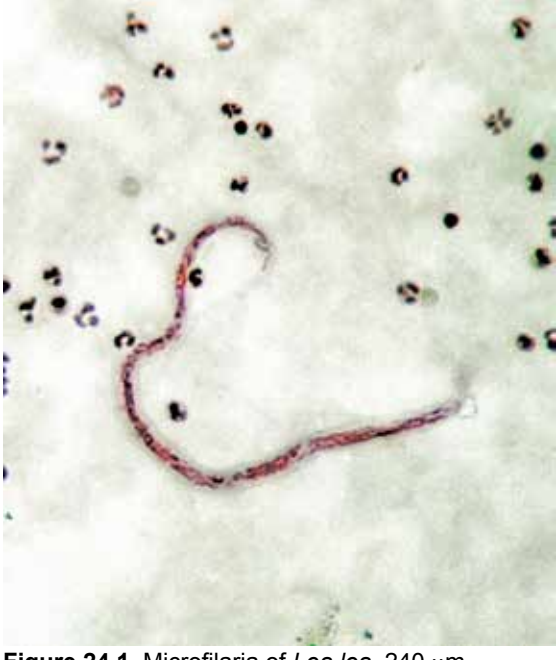


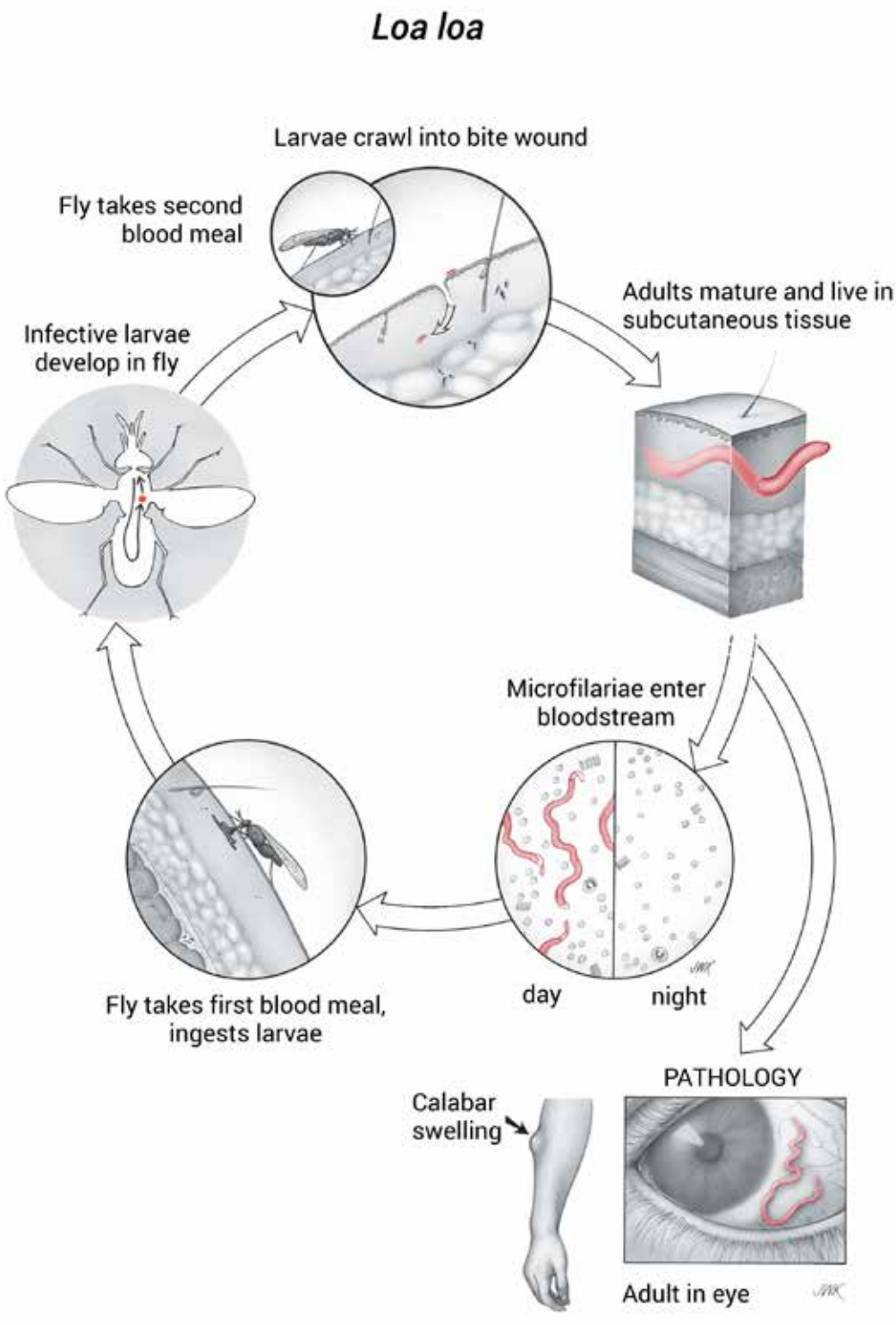
Figure 24.1. Microfilaria of *Loa loa*. 240 μm.

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.[5] 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.[6] In 1890, Stephen McKenzie, an ophthalmologist, found microfilariae and sent them to Patrick Manson suggesting that they might be the larvae of *Loa loa*.[7] In 1895, Douglas Argyll-Robertson published the first complete description of the worm and the clinical presentation of the infection.[8] 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.[6] In 1910, Patrick Manson, along with his colleague George Low, suggested that the swellings were directly connected with infections due to *Loa loa*.[9, 10] These inflammatory lesions are still referred to as Calabar swellings. In 1913, Robert Leiper, described two species of dipterans, *Chrysops dimidiata* and *C. silacae,* as the vectors of *L. loa*.[11]

Life Cycle

Female adults measure 60 mm by 0.5 mm; the males are 32 mm by 0.4 mm.[12] Adult worms deposit microfilariae (Fig. 24.1), while they wander throughout the subcutaneous tissues. There is an interval of 6–12 months between the initial bite by an infected fly and the appearance of microfilariae in the blood. Microfilariae (80 μm by 7 μm) penetrate capillaries and enter the bloodstream, where they circulate until they become ingested in


Loa loa
Larvae crawl into bite wound
Fly takes second blood meal
Adults mature and live in subcutaneous tissue
Infective larvae develop in fly
Microfilariae enter bloodstream
Fly takes first blood meal, ingests larvae
day
night
PATHOLOGY
Calabar swelling
Adult in eye

a blood meal by a *Chrysops* spp. These flies are commonly called deer flies, horse flies, yellow flies, stouts, mango flies, or mangrove flies.[12-14]

L. loa microfilariae exhibit diurnal periodicity that coincides with the feeding habits of *Chrysops*.[15] 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.[16]

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 native to, and living in, endemic areas there is evidence for immune tolerance with high levels of circulating microfilariae, low levels of filarial-specific IgG, and few if any clinical symptoms.[17]

In temporary residents there can be peripheral eosinophilia, high levels of filarial-specific IgG, eosinophil activation and degranulation, and localized subcutaneous edema.[17, 18] The localized swelling referred to as Calabar swellings are usually restricted to visitors to endemic areas and are not typically seen in residents who live for many years in these same regions.[19, 20] The visitors to endemic areas may have exuberant immune responses with parasite-specific IgG and IgE production as well as proliferation of antigen-specific T cells.[21, 22] These patients 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 in their lives.[23, 24] Unlike other filarial nematodes, there is currently no evidence that *L. loa* contains *Wolbachia* symbionts, based on extensive investigations employing light microscopy, electron microscopy, and PCR.[25, 26]

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 for 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.[20] The most serious and life-threatening reactions result after patients are exposed to antihelmintic 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).[23] 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.[27] While less common in patients with low levels of micro-

filariae in the blood, neurological symptoms have been seen with treatment initiation.[28] A rare cardiac condition known as endomyocardial fibrosis may result from eosinophilic infiltration of the myocardium in response to *L. loa*.[29]

Significant differences in the clinical presentation of loiasis occur among long-term visitors to endemic areas, in contrast to people native to these areas.[21] People living in endemic regions are often asymptomatic, despite high levels of circulating microfilariae, whereas visitors and expatriates suffer from a variety of allergic responses, such as frequent episodes of angioedema, Calabar swellings, hypereosinophilia, and hyper-gammaglobulinemia. This disorder is sometimes referred to as the "hyper-responsive syndrome" of loiasis, reflecting elevated humoral and cell-mediated immune responses to the parasite.[20]

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.[30] 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, the presence of a sheath, and three or more terminal nuclei.[24]

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.[31] Since visitors to endemic areas often have low levels of circulating microfilariae, serology can be useful in this population.[30, 32, 33] Antibodies against *L. loa* of the IgG_4 immunoglobulin subclass may be a reliable marker of infection.[30] This includes IgG_4 antibodies against the *L. loa* recombinant antigen L1-SXP-1.[34] There is also a microsatellite that has been developed to detect microfilariae in blood by PCR.[33, 35] Real-time PCR was developed and then adapted for loop-mediated isothermal amplification (LAMP) as a point of care test.[36, 37]

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.[38] Alternatively, new blood and urine *L. loa*-specific biomarker tests have been developed.[39, 40]

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. This may be accomplished through apheresis or several weeks of treatment with albendazole.[24, 41, 42] 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.[28] These iatrogenic complications of loiasis are rare when the microfilarial concentration in blood is less than 2,500 mf/ml at initiation of therapy.[24] 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.[43]

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.[43]

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.[41] In the Chailu 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.[44, 45] 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 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.[45] 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.[46, 47] 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.[39]

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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\), commonly known as the 'Guinea worm' and sometimes referred to as "the fiery serpent of the Israelites", 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. Through a coordinated global eradication campaign spearheaded by a coalition that included the World Health Organization, the Centers for Disease Control and Prevention (CDC), and the Carter Center in Atlanta, Georgia, as well as other agencies, the prevalence of Guinea worm infection has fallen. Due to these efforts, in 2014 the total number of reported cases had decreased from over 3 million to just 126.[3-5] In 2017, according to the Carter Center, only 30 cases were reported worldwide with transmission restricted to Chad, Ethiopia, and Mali.

Figure 25.1. *Dracunculus medinensis*. The large circular blister, from which the worm is emerging, will heal leaving a disfiguring scar.

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. Even in the Sudan former President Jimmy Carter was able to negotiate a several month-long ceasefire 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 has promoted poverty in developing countries. Humans are the primary, and in most areas only, reservoir for *D. medinensis*. There has been significant confusion regarding the existence of 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* can, in fact, infect dogs.[9] The presence of Guinea worm in dogs in Chad may possibly be sustaining transmission in that country.[8, 10, 11]

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 removal of the adult female by slowly wrapping it around a stick to extract

Dracunculus medinensis

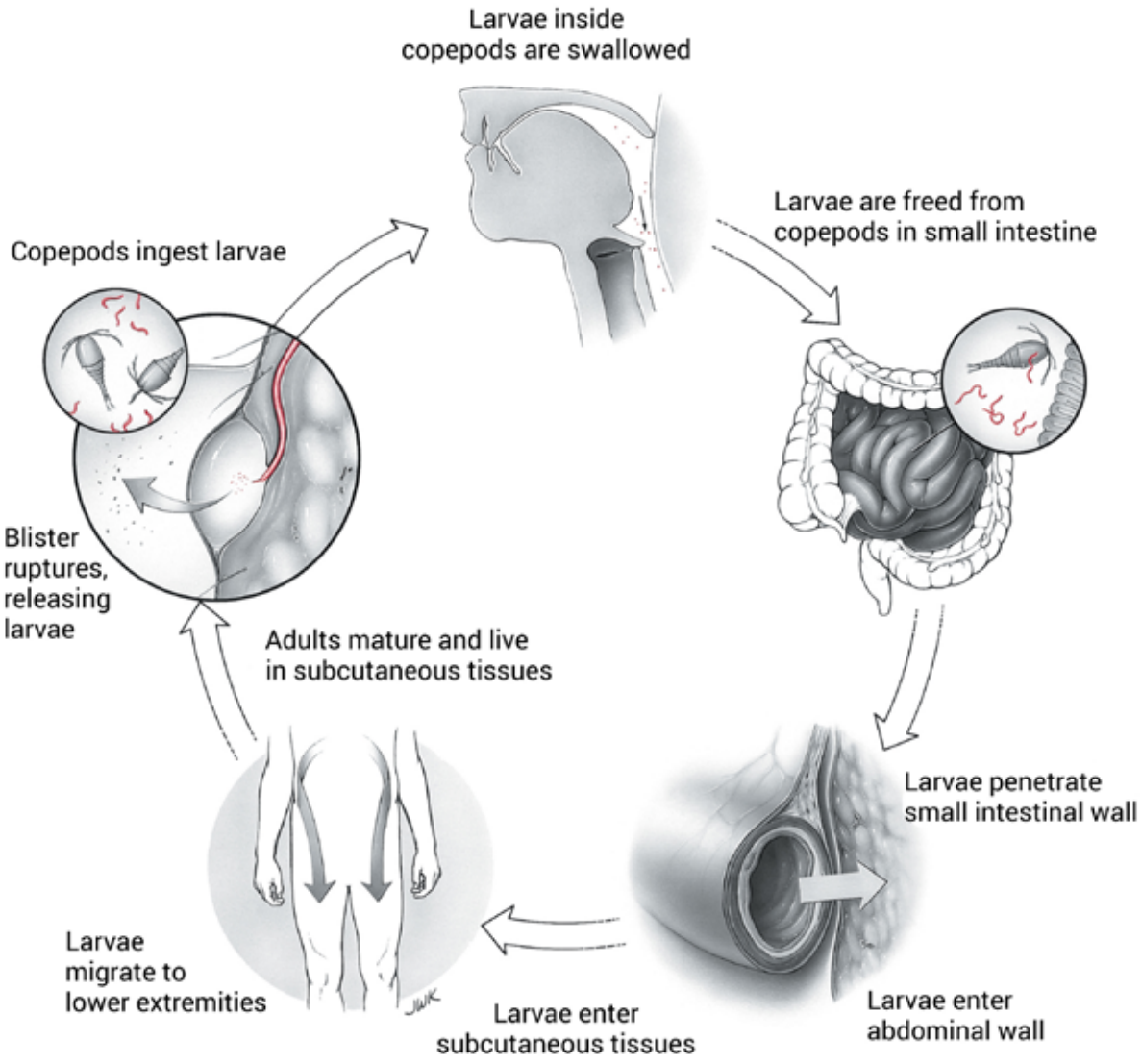

Figure 25.2. Removing an adult *Dracunculus medinensis*. By CDC - This media comes from the Centers for Disease Control and Prevention's Public Health Image Library (PHIL), with identification number #1342.

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 as a symbol of individuals involved in the healing arts.[14] Dracunculiasis is described in the Bible as the fiery serpents 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, Aleksej 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 not known.[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, usually in drinking water, swallow copepods infected with L3 larva.

The copepods are digested in the small intestine, releasing the infective 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 to adults. Approximately one year after ingestion of an infected copepod, the fertilized female Guinea worm finishes her migration to the subcutaneous tissues and induces the formation of a papule that evolves into a fluid-filled vesicle or blister. This vesicle forms in such a way that it surrounds the worm's anterior vulva. The vesicles usually develop in the lower extremity, particularly the foot, but can also develop in a number of other locations.[21] A severe burning sensation develops at the location of this vesicle, prompting the afflicted individual to submerge the limb or foot into water in an attempt to find relief from the pain.[14] When the vesicle comes in contact with freshwater, 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] Larvae are ingested by copepods of many genera, including *Cyclops*, *Mesocyclops*, and *Thermocyclops*.

The larvae rapidly penetrate the hemocele

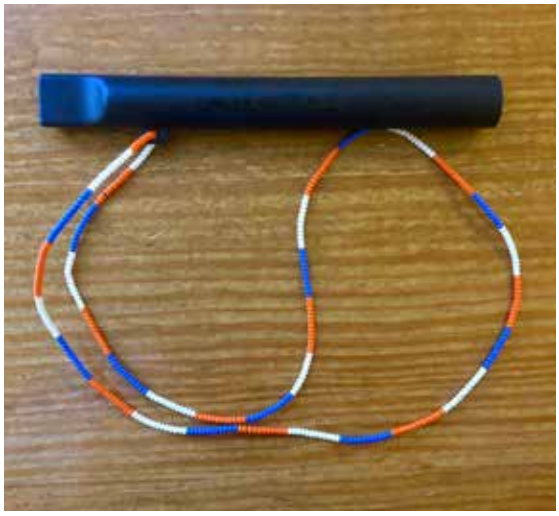

Figure 25.3. Filter straw used to strain infected copepods out of drinking water to prevent infection.

of the crustacean and develop within 2–3 weeks into infective L3 larvae. The life cycle is completed when a human host ingests these infected crustaceans. In other disease-endemic countries, particularly Chad, dogs may serve as an important reservoir.[10, 11]

Cellular and Molecular Pathogenesis

During a 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 results in the formation of an ulcer. Just prior to 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 secretions of the worm, 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* (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 a fatal disease, but it 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. In some cases, ankylosing of joints, arthritis, or contractures can develop, and lead to permanent disability.[25] At one time in Nigeria, it was estimated that Guinea worm infection in adults resulted in an average of 100 days of work lost per year.[26]

Diagnosis (see Clinical Appendix)

Definitive diagnosis is achieved by locating the head (end with the vulva) 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 totally extracted (Fig. 25.2). This must be done slowly to ensure that the worm does not break. Surgical removal of the worms has been effective, but may exaggerate allergic reactions. Mebendazole treatment or use of other antihelmintic therapy of dracunculiasis is not recommended.[28] Wound care and pain management are important components of the care of 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, 5. the methods for controlling transmission are relatively simple.[29, 30] Recently, however 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, a complication that 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 in order 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 ever achieved by a former U.S. President.

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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, modes of infection, methods of diagnosis, 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-ˈlar-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. This parasite is transmitted by the bite of an infected mosquito and is not transmitted directly from person to person or dog to person.[2]

There is concern that climate change is driving an increase in the incidence of *Dirofilaria* spp. in animals as well as humans.[2] A number of cases have been identified in the United States, particularly in Texas, Florida, and Louisiana, but 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 either excretory-secretory products or somatic antigen from the adult worm.[4] *Dirofilaria tenuis*, a parasite of raccoons and related *Dirofilaria* spp., cause 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 eosinophilia.

Capillaria hepatica
(Bancroft 1893)

Pronunciation: \kap-ə-ˈlar-ē-ə\\ hi-ˈpa-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, particularly the rat, that can on rare occasion infect humans.[7] Human cases have been reported in Asia, South America, North America, Eastern and Western Europe.[7, 8] Adult worms live and lay eggs in the liver parenchyma within a syncytium. These eggs are not released into the environment until 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 to adults and reach the liver. After mating, they begin to lay eggs, completing the life cycle. The liver serves as the source of nourishment for adults. If enough parasites are present, the host may suffer liver failure and die. Rarely this pathogen can be transmitted to humans if they accidentally ingest embryonated eggs.

Previously, diagnosis required liver biopsy and the visualization of adults or eggs in the samples, but now serological testing is available.[9] Often the disease is unsuspected and discovered only at autopsy or incidentally

through liver biopsy. Alternatively, it can present 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 passage through the intestinal tract of ingested eggs, as true infection does not result in egg release into the feces. (Fig. C.55) Numerous treatment regimens, including disophenol (2-6-diiodo-4-nitrophenol), albendazole and prednisone have been used successfully in management of this infection.[11-13]

Capillaria philippinensis

(Chitwood, Velasquez, and Salazar 1968)

Pronunciation: \kap-ə-ˈlar-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] A number of 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 those of *Trichinella spiralis* in both 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, there is no evidence that patients harboring *C. philippinensis* are immunosuppressed. As infection progresses, the patient first begins passing embryonated, then unembryonated eggs in the stools.

Figure 26.1. Adults of *Capillaria philippinensis*. The female is 3 mm x 45 μm, and the male is 2.5 mm x 30 μm.

C. philippinensis is most likely a parasite of waterfowl that feed on fish, which are 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 vegetables and a variety of live aquatic animals including shrimp, is thought to be a common source of this infection.

The clinical disease consists of a 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 more than 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

Capillaria philippinensis

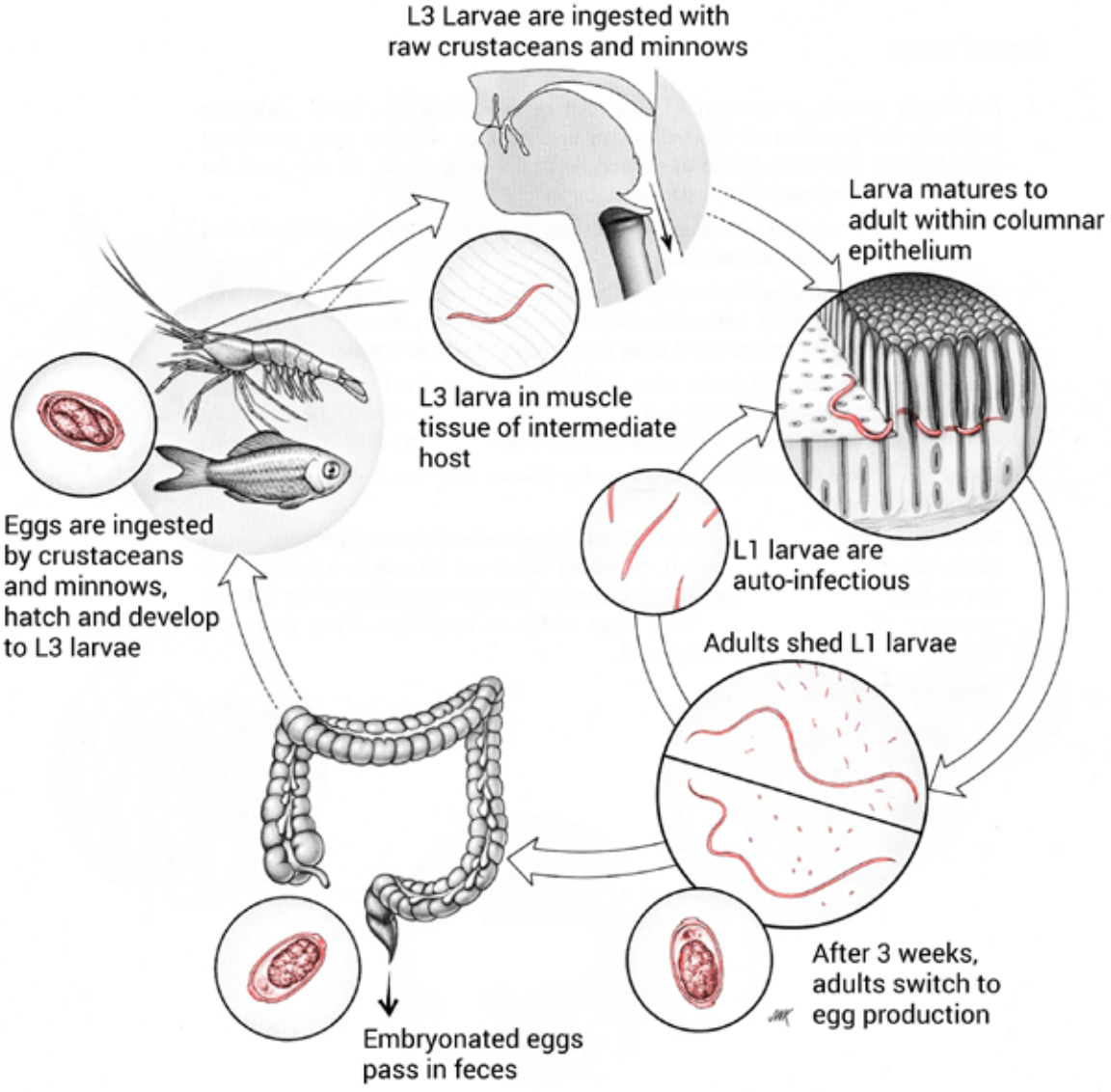

Table 26.1. Other nematodes of medical importance

Parasite	Geographic distribution	Major pathologic consequences	Mode of infection	Diagnosis
**Capillaria hepatica*	Worldwide	Necrotic lesions in liver	Oral route	Biopsy
**Capillaria philippinensis*	Philippines	Malabsorption syndrome, diarrhea	Oral route	Stool examination for larvae and eggs
Dioctophyma renale	North and South America, China	Complete destruction of infected kidney	Oral route	Urine examination for eggs
**Dirofilaria immitis*	Worldwide	Pulmonary nodules	Bite of infected mosquito	Biopsy
**Mansonella ozzardi*	Central and South America, Caribbean	Chronic arthritis	Bite of infected midge	Blood smear or Knott's test for microfilariae
**Mansonella perstans*	Central and South America, Africa	None	Bite of infected midge	Blood smear or Knott's test for microfilariae
**Mansonella streptocerca*	Africa	Pruritic dermatitis	Bite of infected midge	Impression smear of skin snip for microfilariae
**Oesophagostomum bifurcum*	West Africa	Intestinal nodules	Oral route	Stool examination for eggs
Syngamus laryngeus	South America, Caribbean, Philippines	Asthma, hemoptysis	Unknown	Sputum or stool examination for eggs
**Ternidens diminutus*	Africa	Iron-deficiency anemia	Unknown	Stool examination for eggs
Trichostrongylus spp.	Worldwide	Anemia	Oral route	Stool examination for eggs

* Discussed in the text.

some endemic areas. Numerous *C. philippinensis* worms can be identified at all stages in the lumen, and in the intestinal mucosa at autopsy. As many as 200,000 worms have been recovered in 1 liter of bowel fluid, a consequence of autoinfection.[6]

Diagnosis depends upon finding eggs or larvae in feces, or detecting the parasite on biopsy of the small intestine.[16] They are usually present in patients presenting with abdominal pain, diarrhea, and weight loss.[6] Eggs bear some resemblance to 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 as well as 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 to propagate the life cycle in fish and other intermediate hosts. Avoidance of raw or undercooked fish is recommended to prevent infection. Cultural eating habits are, however, extremely difficult to change.

Mansonella ozzardi

*(*Manson 1897)

Pronunciation: \man-sə-ˈnel-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 locate to the visceral adipose tissue, the peritoneal or thoracic cavities, or even the 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 asymptomatic 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. Serological 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 significantly reduce the number of circulating microfilariae and reduce symptoms.[23-26] Neither diethylcarbamazine citrate (DEC) nor benzimidazoles are effective against *M. ozzardi*.[24, 27]

Mansonella perstans

(Manson 1891)

Pronunciation: \man-sə-ˈnel-ə\\pər-stans\

Mansonella perstans (MAN-so-nel-ah) is a filarial parasite found in Africa, and in 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 from rural Senegal.[28] The adults live free in serous cavities such as the pleural, pericardial, or peritoneal cavities, where they produce 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 that are 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 examination of a stained blood smear or by the Knott test. Serological testing is available but lacks specificity. A PCR test has been developed but is not widely available.

Most standard antihelmintic therapies have been ineffective for *M. perstans*.[30-32] The discovery that this filarial parasite harbors *Wolbachia* endosymbionts 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 the endosymbiont *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-ˈnel-ə\\strep-tə-ˈsər-kə\

Mansonella streptocerca (\MAN-so-nel-ah\\\ STREP-tow-ser-ka\) is a filarial parasite with a distribution restricted to tropical rainforests in Central Africa. Adult worms locate to the subcutaneous tissues, as do microfilariae.[39, 40] Biting midges are its vectors. The major clinical manifestation of this infection 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 that can aid in detection of the 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, there are some concerns about side effects.[43, 44]

Oesophagostomum bifurcum
(Creplin 1849)

Pronunciation: \i-ˌsäf-ə-ˈgäs-tə-məm\

Oesophagostomum bifurcum (I-sof-ah-GOS-tow-mum) is a nematode mostly infecting non-human primates in Africa and Asia. In northern Togo and northeastern Ghana, *O. bifurcum* infects up to 30% of these human populations, with an estimated 250,000 cases.[45-48] Sporadic cases have also been described in other parts of Africa, Asia and South America.[47] In these regions, adults 30–40 years of age have the highest prevalence. 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 to 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 to 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 abdomen. Often the mass is asymptomatic. As a result, the infection is frequently diagnosed at biopsy, although ultrasound is also of great value.[50] Fecal examination is the diagnostic method of choice in individual patients (eggs cannot be visually distinguished from hookworm eggs), while 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-ˌdenz\

Ternidens diminutus (ter-nah-DENZ) is a nematode capable of infecting humans that resembles *O. bifurcum*. *Ternidens* eggs resemble hookworm eggs, so that *T. diminutus* is sometimes referred to as "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]

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27. Aberrant Nematode Infections

Many nematodes are zoonotic and only occasionally infect humans. These "aberrant" nematodes are incapable of maturing to adult parasites in the human body. Cutaneous larva migrans (CLM) and visceral larva migrans (VLM) are two diseases caused by this type 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-ˈläs-tə-mə\) and *Uncinaria stenocephala* (\un-sin-NAR-EE-ah\\ste-NO-seh-FAAL-ah\, \ən-sə-ˈnar-ē-ə\\ste-nō-sə́ˈfāl-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-ˈä-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 are permitted to 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 were 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 antihelmintic 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 and for this reason many experts recommend that this should not be performed.[7]

Visceral Larva Migrans

Toxocara canis
(Johnston 1916)

Toxocara cati
(Brumpt 1927)

Pronunciation: \täk-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 serious condition than CLM.

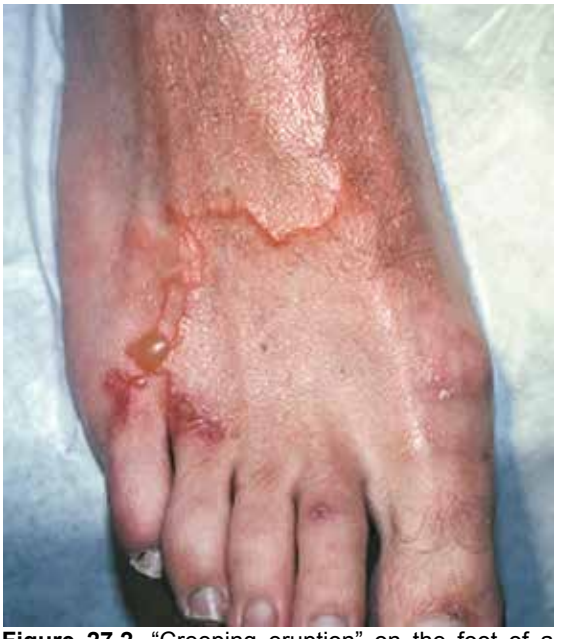


Figure 27.2. "Creeping eruption" on the foot of a patient who stepped on an infective larva of *A. braziliense*. Courtesy G. Zalar.

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 a high circulating eosinophilia, and suffered severe multisystem disease caused by *T. canis* and *T. cati* larvae.[11] Both *T. canis* and *T. cati* have a life cycle in their respective hosts resembling that of *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, infection begins with ingestion of embryonated *Toxocara* eggs (Fig. 27.4). This commonly occurs where children are playing in contaminated sandboxes and playgrounds.

Table 27.1. Cutaneous larva migrans: clinical manifestations

Organism	Predominant location in body	Major pathologic consequences	Diagnosis
Ancylostoma braziliense	Skin	Urticaria, serpiginous lesion	Biopsy
Uncinaria stenocephala	Skin	Urticaria, serpiginous lesion	Biopsy
Strongyloides procyonis	Skin "Duck-hunter's itch"	Urticaria	Biopsy
Dirofilaria conjunctivae	Palpebral conjunctivae	Abscess formation subcutaneous tissues	Biopsy
Dirofilaria repens	Subcutaneous tissues	Fibrotic, painless nodule formation	Biopsy
Anatrichosoma cutaneum	Subcutaneous tissues	Serpiginous lesions	Biopsy
Rhabditis niellyi	Skin	Papule, urticaria	Biopsy
Lagochilascaris minor	Subcutaneous tissues around head and neck	Abscess formation	Biopsy
Gnathostoma spinigerum	Subcutaneous tissues	Abscess formation	Biopsy, ELISA
Thelazia callipaeda	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

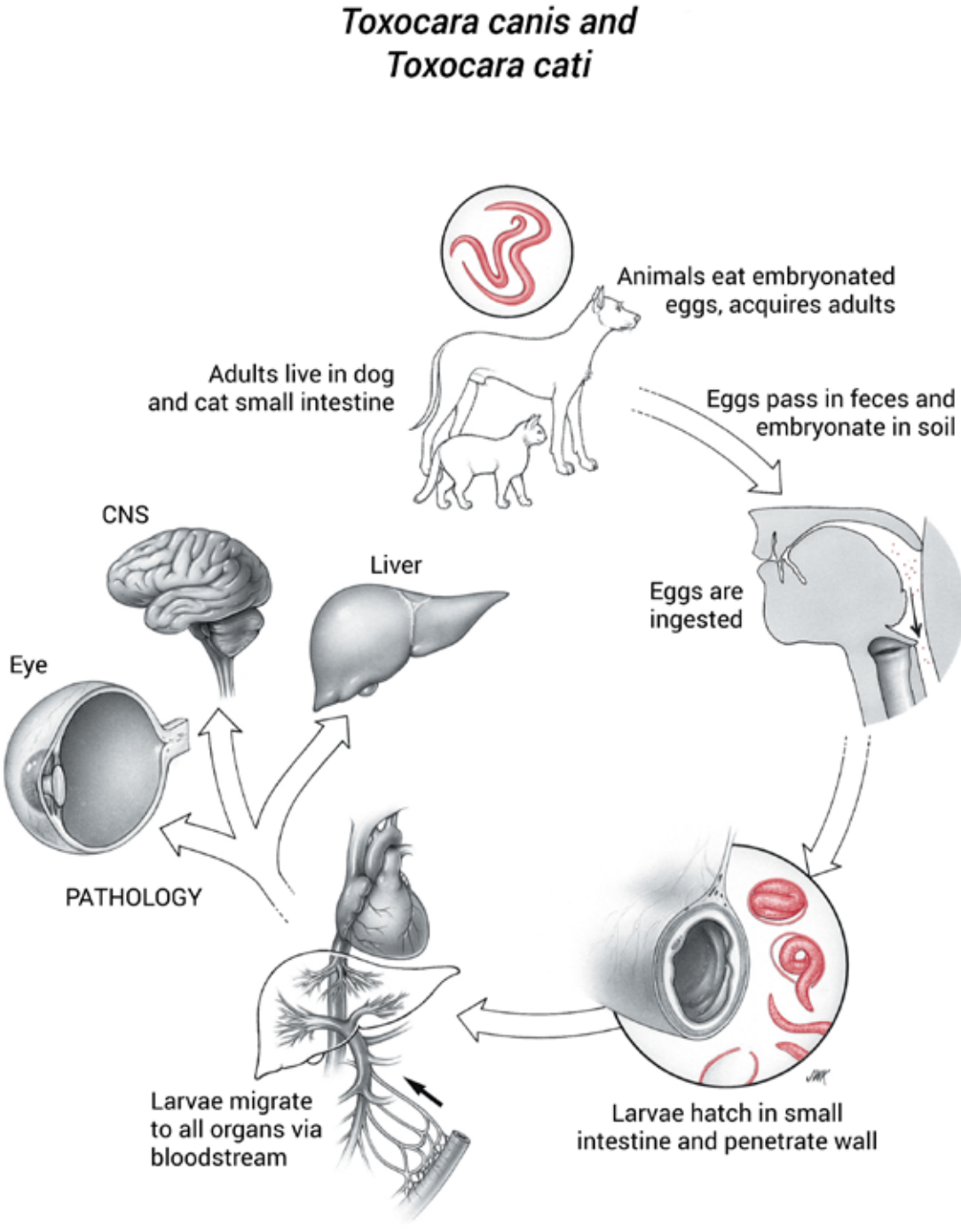
Organism	Predominant location in body	Major pathologic consequences	Diagnosis
Toxocara cati	Viscera, eye	Blindness	ELISA
Toxocara canis	Viscera, eye	Blindness	ELISA
Baylisascaris procyonis	Meninges	Meningoencephalitis	CSF examination
Angiostrongylus cantonensis	Meninges	Meningoencephalitis	CSF examination
Angiostrongylus costaricensis	Mesenteric arterioles	Peritonitis	Biopsy
Anisakis spp.	Stomach wall	Granuloma	Biopsy
Phocanema spp.	Stomach wall	Granuloma	Biopsy
Terranova spp.	Stomach wall	Granuloma	Biopsy
Oesophagostonum spp.	Small and large intestinal wall	Granuloma	Biopsy
Gnathostoma spinigerum	Striated muscles,subcutaneous tissues,brain, small intestinal wall	Abscess, meningoencephalitis	Biopsy, ELISA
Dirofilaria immitis	Lung, heart	Granuloma in lung	Biopsy

ELISA: enzyme-linked immunosorbent assay; RIA: radioimmunoassay; CSF: cerebrospinal fluid.

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 into 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, after weeks to months of migration, the larvae die, 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 itself was responsible for the loss of sight.[16, 17]

Epidemiologic evidence suggested that ocular disease tended to occur in the absence of systemic involvement and *vice versa*, which led


Toxocara canis and Toxocara cati
Animals eat embryonated eggs, acquires adults
Adults live in dog and cat small intestine
Eggs pass in feces and embryonate in soil
CNS
Liver
Eye
Eggs are ingested
PATHOLOGY
Larvae migrate to all organs via bloodstream
Larvae hatch in small intestine and penetrate wall

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, whereas 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 fever, enlargement of the liver and spleen, lower respiratory symptoms (particular bronchospasm, resembling asthma), eosinophilia (sometimes approaching 70%), and hypergammaglobulinemia of IgM and IgG classes.

Figure 27.3. Adults of *Toxocara canis*. The female is 9 cm, the male is 6 cm.

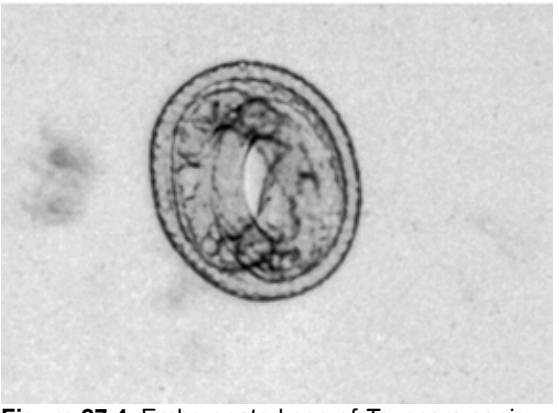


Figure 27.4. Embryonated egg of *Toxocara canis*.

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 an eosinophilic meningoencephalitis.[21]

There has been an increasing appreciation that subtler clinical manifestations might also arise as a result of larval migrations. So-called "covert toxocariasis" ranges in spectrum 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 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

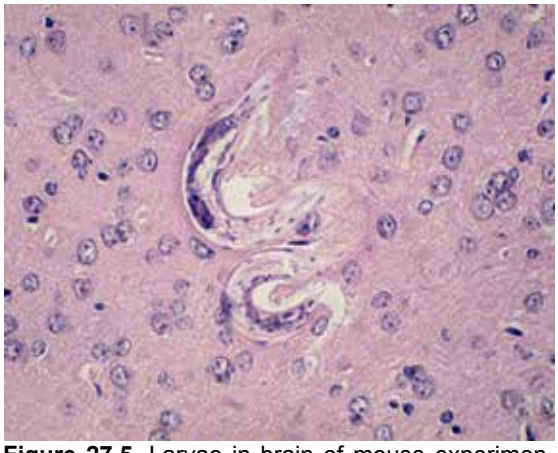


Figure 27.5. Larvae in brain of mouse experimentally infected with *T. canis*.

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]

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.[33] The most serious consequence of the infection is invasion of the retina, leading to granuloma formation, which occurs typically peripherally, or in the posterior pole. These granulomas drag the retina and create a distortion or detachment of the macula.[34] The degree of visual acuity impairment depends upon the specific 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 retinoblastoma there is the risk of unnecessary enucleation of the infected eye.[35-38]

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 and history of pica make the diagnosis of VLM more likely. Similarly, OLM should be suspected in any child with unilateral vision loss and strabismus. Diagnostic tests for VLM are primarily immunological.[39]

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.[40, 41] Newer diagnostics are also under development including those that use recombinant antigens in place of parasite natural products.[42, 43] 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.

Other indicators include hypergammaglobulinemia and an elevated isohemagglutinin titer. A constellation of clinical disease

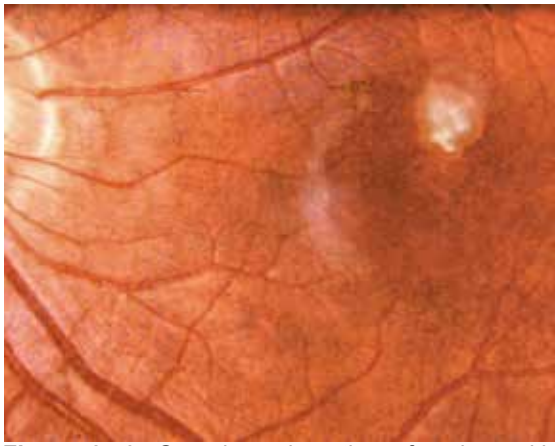


Figure 27.6. Granuloma in retina of patient with OLM. The lesion consists largely of eosinophils.

described above, 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 on the basis of 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.[44]

Treatment (see Clinical Appendix)

While there is limited reliable evidence-based guidance with regard to treatment, *T. canis* and *T. cati* can be treated with albendazole 400mg oral 2x/day for a total of 5 days (adult and pediatric dose).[32] The other commonly used drug, mebendazole, is poorly absorbed outside the gastrointestinal (GI) tract, although some success has been reported in patients who ingest 1g or more of this agent for a 21-day course.[45] Symptomatic treatment, including administration of corticosteroids, has been helpful for suppressing intense manifestations of the infection. OLM is treated either by surgery (vitrectomy), antihelmintic chemotherapy, and/or corticosteroids.[46-49] In the case of ocular involvement with active ocular inflammation, the role of antihelmintic therapy is unclear, owing to the lack of knowledge regarding the intra-ophthalmic pharmacokinetics and 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.[46] 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.[47] 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, 50-54]

Baylisascaris procyonis

(Sprent, 1968)

Pronunciation: \bā-lis-as-kə-rəs\\pro-sī-ən-nəs\

Baylisascaris procyonis (\BAY-lis-as-KA-RIS\\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.[39, 41, 44] Although it is rare, the pathological con-

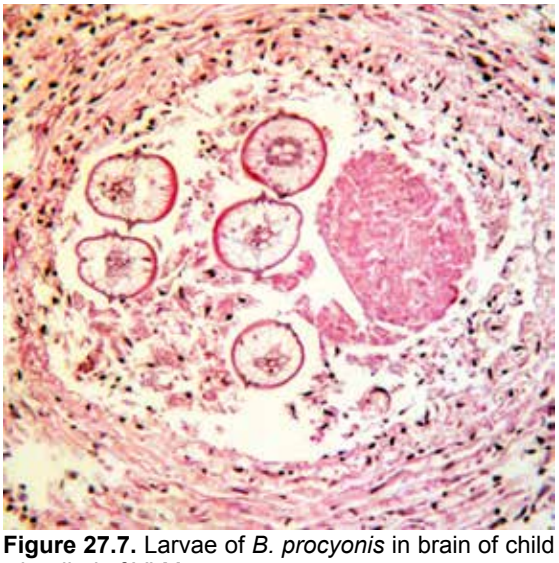


Figure 27.7. Larvae of *B. procyonis* in brain of child who died of VLM.

sequences 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 anti-helmintic chemotherapy and steroids.[55] The large, developing larvae (up to 2 mm.) cause considerable mechanical damage.[55] 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.[56]

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.[57] Although a serological test has been developed for *B. procyonis* that has 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 antihelmintic 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[58-60]

Angiostrongylus cantonensis
(Chen 1935)

Pronunciation: \an-jē-ə-ˈsträn-jə-ləs\\kan-tən-ən-səs\

Angiostrongylus costaricensis
(Morera and Cespedes 1971)

Pronunciation: \an-jē-ə-ˈsträn-jə-ləs\\ kȯ-stə-ˈrē-kən- səs\

Angiostrongylus cantonensis (\AN-jee-o-STRON-jah-lus\\KAN-ton-en-SIS\) infection occurs in Southeast Asia, the Philippine Islands, Taiwan, and both North and South Pacific islands including Hawaii. Human infection was first described in Taiwan when larvae of the parasite were isolated from the CSF of a child. Infected rodents, but not human cases, have also been reported from East Africa. A survey of wharf rats in New Orleans revealed a high rate of infection, although none had been detected several years prior to that study.[52] Although *A. cantonensis* is present in all the main Hawaiian Islands, the eastern portion of the Big Island of Hawaii is the epicenter for this infection paralleling the introduction of the semi-slug *Parmarion martensi*.[61] Acquisition of human infection depends upon the locale and the nutritional habits of the specific population. A case in Hawaii resulted from ingestion of a raw slug given for a traditional medicinal purpose. In Tahiti, epidemics have resulted from consumption of raw freshwater prawns, and elsewhere, other invertebrates (e.g., planarians) either consumed directly or in vegetation.

Rats are the definitive host for *Angiostrongylus* spp. *Angiostrongylus* infect several species of wild rats including the common wharf rat, *Rattus norvegicus*. These worms live, and lay eggs in, the pulmonary arteries of rats. The released eggs move through the capillaries in the lung, and then penetrate into the alveolar spaces. From the alveolar spaces, the larvae migrate up the respiratory tree and are swallowed, ultimately to pass out of the rat in feces.[62] *A. cantonensis* larvae incubate in the soil before being consumed by a variety of mollusks and crustaceans.[63-66] Humans are infected when they inadvertently consume: these slugs; 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 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 has been observed in infants and small children. These nematodes die surrounded by an eosinophil-rich inflammatory infiltrate.[67] 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 eosinophilic 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.[68] Vomiting is reported in the majority of cases, as are migrating painful paresthesias.[69] 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.[70]

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.[67] The natural course of the disease without treatment is for resolution of symptoms in 1–2 weeks, but disease duration and severity may be worse 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. [71-73]

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.[69] PCR testing is available and can be performed on CSF but lacks high sensitivity.[74]

With the self-limiting nature of this disease, treatment often focuses on symptoms rather than on antihelmintic therapy. Although animal models have supported the use of antihelmintic therapy trials, efficacy in human infection has not been demonstrated, and there are reports that antihelmintic therapy may lead to more serious disease.[75-77] When antihelmintics 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.[51]

Gnathostoma spinigerum

(Owen 1838)

Pronunciation: \nə-ˈthäs-tə-mə\\spi-ni-ge-rəm\

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.[55, 60, 78]

Female adults are 25–54 mm in length, while males measure 11–25 mm. Adults live coiled in the wall of the stomach of their definitive hosts. Eggs pass in feces and hatch in water, releasing larvae that are ingested by macroinvertebrate crustaceans. Host vertebrates eat infected crustaceans and the infective stage for humans then develops within them. When humans eat these infected vertebrates, the larvae invade the small intestinal tissue. The worms leave the intestine and migrate into the deep tissues.[79-81]

Although the disease is likely 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* is also able to invade the eye and result in an intraocular form of this disease.[82]

Previously the diagnosis of gnathostomiasis was limited to an appropriate travel history, clinical presentation and 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 isolation of the parasite is often not possible.[83]

Although this disease can be self-limited, therapy often involves a 21-day oral course of albendazole at a dose of 400mg once daily.[84] Ivermectin has been shown to be effective in several trials, and may serve as an alternate agent. It remains unclear as to the role of antihelmintic therapy in ocular and neurological disease where it may worsen symptoms. In some cases, surgical removal is employed.[55] Corticosteroids have been used in the treatment of patients with CNS manifestations. Relapses have been reported with either therapy.[69]

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-ˈsā-kəs\), *Phocanema*, *Terranova* (\TEAR-ah-now-vah\,\ter-ə-ˈnō-və\), and *Contracaecum.* They infect sea mammals, particularly dolphins, whales, sea lions, and seals.[85] In these hosts, adults live in the lumen of the stomach. Anisakid L2 larvae infect a number of crustacean species. L3 larvae infect a wide variety of bottom-feeding fish.[86]

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. There they mature into the stage infective for fish, as well as squid; paratenic (transport) hosts that serve to 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, where they grow up to 3cm in length. Upon the death of the cold-blooded vertebrate host, larvae migrate to their muscles where they may be ingested by marine warm-blooded 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.[87]

Raw or undercooked saltwater fish, often in the form of sushi or sashimi, has become a popular style of cuisine throughout the world.[56, 57] 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. Tissue invasion is facilitated by release of parasite hydrolytic enzymes.[88, 89] Infected individuals often present with abdominal pain that

can be severe, and can be confused with the symptoms of an acute gastric ulcer.[90] From ingestion to onset of symptoms is usually a matter of minutes to hours. Nausea, vomiting, abdominal distension, mild fever and diarrhea with blood and mucous in the stool can be part of the presentation.[91] If larvae are able to penetrate the mucosa, angioedema, urticarial and allergic symptoms can dominate the clinical presentation.[92]

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 is 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%.[93] PCR tests have been developed, but are not commercially available.[94, 95]

Thorough cooking or freezing of seafood prior to ingestion can prevent infection by anisakid nematodes. Most sushi restaurants in the United States and elsewhere now inspect pieces of raw fish carefully prior to serving them, and the FDA under food code 101 requires freezing prior to 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]

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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 of which are parasitic in the gut tracts of various vertebrate hosts. Tapeworms are flat, segmented worms, composed of a head (scolex), and a series of segments, known as proglottids. Together, all proglottids are 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 the attachment process. The scolex contains nerves terminating in ganglia, while the segments contain only nerves. The neck region of the scolex is metabolically active and is the site in most tapeworms from which new proglottids form.

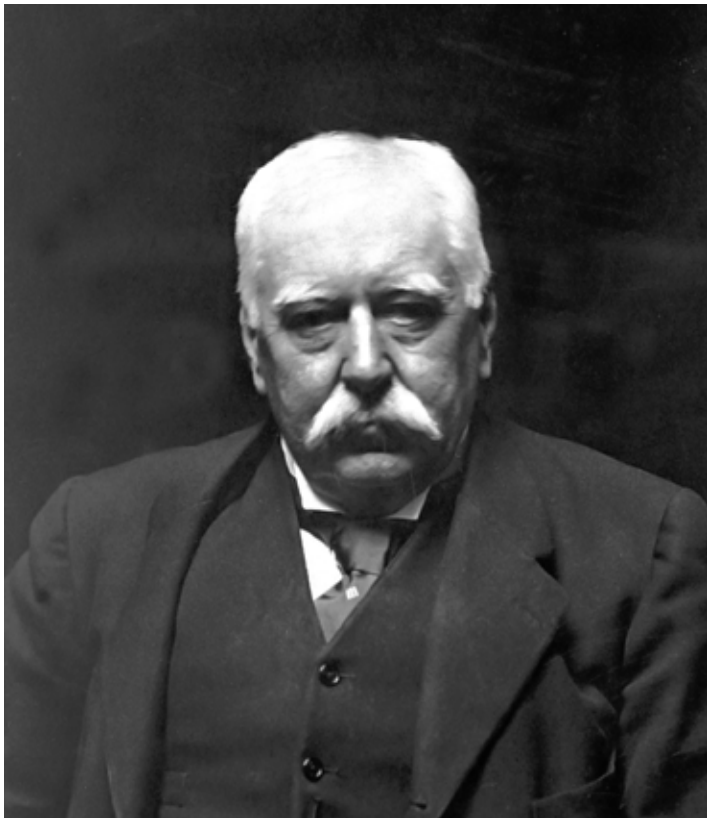
Tapeworms do not have a functional gut tract. Rather, the segments are enclosed in a specialized tegument, whose structure and function are directly related to nutrient acquisition. Evenly spaced microvilli cover the entire surface of the tegument, underneath which lie mitochondria, vesicles (perhaps involved in tegument replacement), and related structures. The tapeworm obtains some of its nutrients by actively transporting them across the tegument. Each proglottid absorbs a wide variety of low-molecular-weight substrates, but its precise metabolic requirements have yet to be fully defined.

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 the segment 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 they are all connected by a common nervous system emanating from central ganglia located in the scolex. Osmoregulation and excretion of wastes occurs 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 mature proglottids that lie next to each other. 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, and contain a hexacanth larva referred to as 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. When this stage is ingested by the definitive host 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, the adult cestodes do not adversely impact childhood development. However, when humans serve as intermediate hosts, the cestodes become significant causes of global morbidity. For instance, neurocysticercosis caused by the larval stages 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 mansoni* (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-nä-tə\

Introduction

Taenia saginata (\TEE-nee-ah\\SA-je-not-u\) belongs to the order Cyclophyllidea, and is one of the largest parasites infecting humans, often achieving lengths approaching 8–10 m. Like all other adult tapeworms, it lives in the lumen of the upper half of the small intestine. There are no reservoir hosts for *T. saginata*. This tapeworm occurs wherever cattle husbandry is prevalent, and where human excreta are not disposed of properly.[1,2] It is commonly referred to as the "beef tapeworm", although the adult sexual stage of the parasite lives exclusively in humans and should perhaps be more properly called the "human tapeworm". Endemic foci include vast regions of Sub-Saharan grasslands in Africa, particularly in Ethiopia, due to the common dietary practice there 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.

Figure 28.1. A rare beef tenderloin.

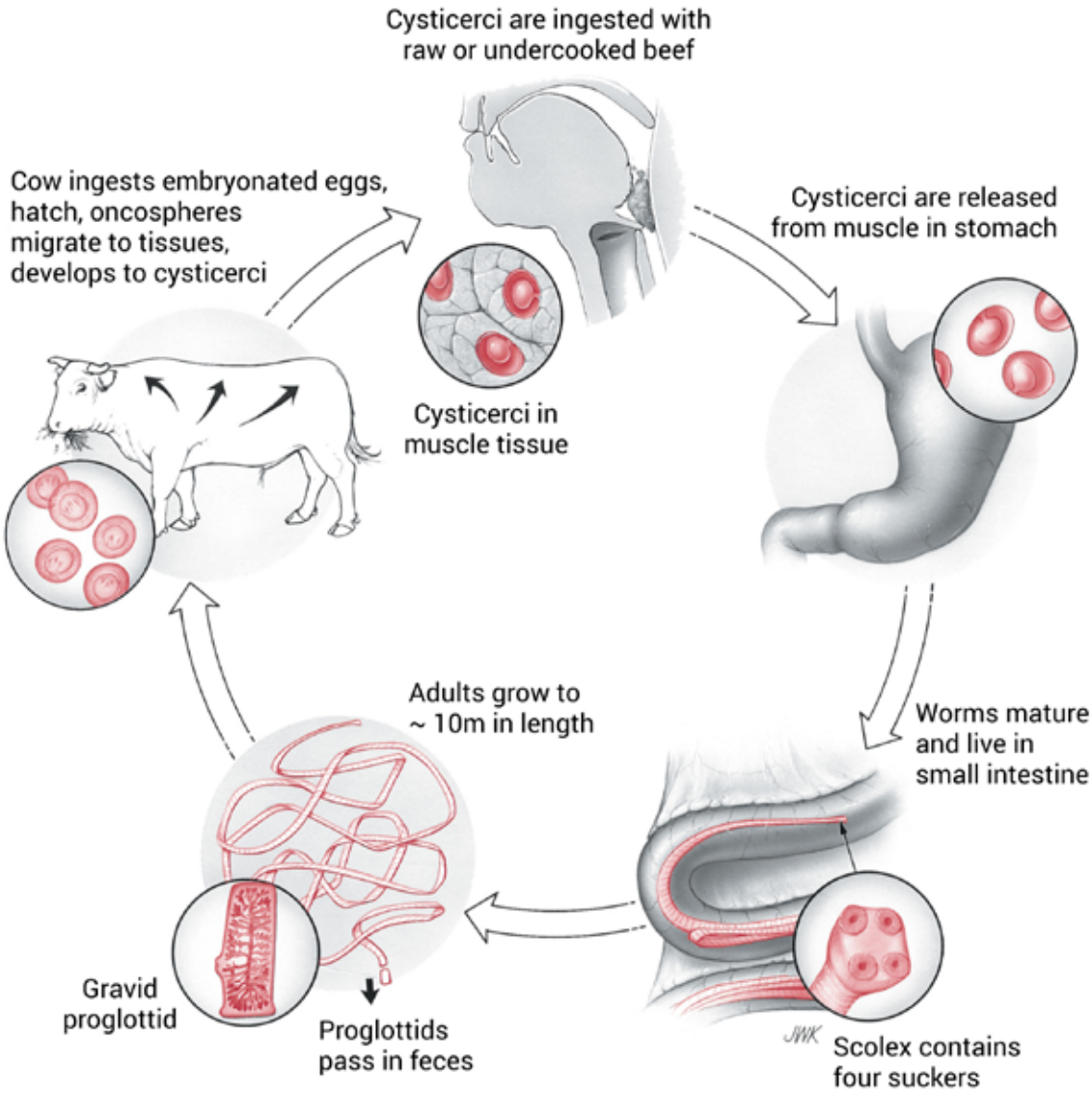
Another *Taenia* spp., *T. asiatica*, infects people in Taiwan, Korea, China, Vietnam, and Indonesia.[3-5] Investigators felt that this organism should be considered a separate species (*T. asiatica*) from *T. saginata*, particularly since the intermediate host is porcine not bovine.[5,6] Molecular tools confirmed that *T. asiatica* is a third species of the *Taenia* genus that infects humans, in addition to *T. saginata*, and *T. solium* ("pork tapeworm", Chapter 29).[7,8] The full clinical spectrum of disease caused by *T. asiatica* is still not fully appreciated.

Historical Information

The history of the different tapeworms is initially intertwined, as it was not until many years after their initial discovery that it was determined that these similar parasites were fundamentally different in terms of their morphology and host specificity.[9] In 1683, Edward Tyson described several species of tapeworms, which he recovered from dogs.[10] Tyson is credited as the first person to recognize the head or scolex of these tapeworms.[9] In 1656, Félix Plater, a Swiss physician, wrote about the distinctions between *Taenia* spp. and *Diphyllobothrium latum* (then called *Lumbricus latus*).[11] Nicolas Andry de Bois-Regard is credited with the first report of *T. saginata* in 1700, but 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 was the first to describe the worm correctly in a larger treatise on helminthology, and suggested that *T. saginata* and *T. solium* adult worms were

Taenia saginata



two different species.[13] In 1784, Goeze indicated that intermediate hosts in the life cycle of tapeworms were required when he noted that the scolices of tapeworms in humans resembled the cysts present in the muscles of pigs.[14] In 1850, Carl Von Siebold speculated that "bladder worms" (i.e., cysticerci) could develop into adult tapeworms, or that perhaps they were only degenerated adults.[15] In 1861, Friedrich Kuchenmeister fed meat from pigs containing tapeworm cysticerci to condemned prisoners, and then recovered the adult worms from these individuals intestines after their execution.[16] In 1863, Friedrich Leuckart reported on experiments showing that the proglottids of *T. saginata*, when fed to young calves, developed into cysticerci (metacestodes) in the animals' muscles.[17] In 1870, John Oliver, demonstrated that after human ingestion of "measly" beef, humans developed adult *T. saginata* infections, and further suggested that thorough cooking of infected meat would prevent infection with the adult tapeworm.[18]

Life Cycle

Infection begins when the cysticercus is ingested in raw or undercooked beef (Fig. 28.1). The cyst enters the small intestine and the wall of the cyst is digested away, freeing the worm inside. The parasite then everts its scolex and attaches to the intestinal wall with the aid of four sucker disks (Fig. 28.2).

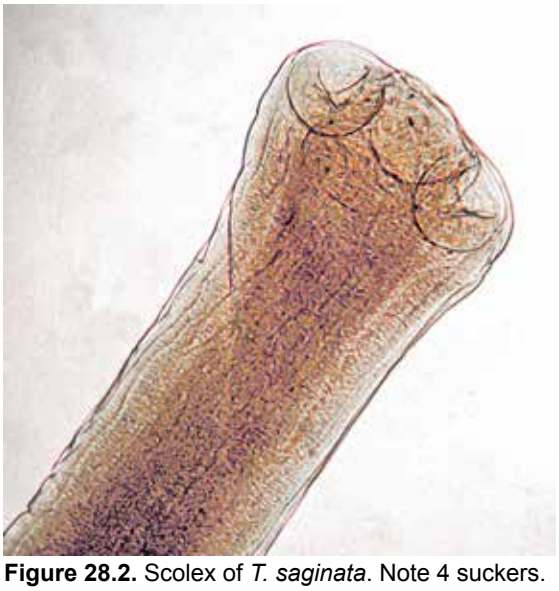


Figure 28.2. Scolex of *T. saginata*. Note 4 suckers.

Figure 28.3. Adult *T. saginata*. Note the position of the scolex (arrow). Courtesy of U. Martin.

A mature adult tapeworm (Fig. 28.3) takes about three months to grow to full length. The developing proglottids (segments) extend down the small intestine, sometimes reaching the ileum. All adult tapeworms feed by actively transporting nutrients (e.g., sugars, amino acids, nucleic acids) across their tegumental surface, since they have no digestive tract. Segments mature as they progress towards the terminal end of the worm. Terminal proglottids, gravid with hundreds of embryonated, infectious eggs, may detach from the colony, and may even 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.

A cow must then ingest a gravid segment, usually along with grass or hay to complete the life cycle. 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 eggs to

hatch in the small intestine (Fig. 28.5). The oncosphere (hexacanth or six-hooked larva) penetrates the intestinal wall, most likely aided by its hooks and secretory peptidases.[19] The larvae enter the bloodstream and are passively carried throughout the body. Oncospheres can lodge in any one of a variety of tissues, depending upon where the circulation takes them. Mostly, they infect striated skeletal muscle tissue. There they encyst and develop to the cysticercus (metacestode). This stage can live 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 being born, and live cysticerci tend not to cause problems, even in neurological tissues (Chapter 32).

It is important to note that cattle cannot become infected with adult parasites if they accidentally ingest cysticerci. Similarly, humans cannot harbor the metacestode, since *T. saginata* eggs will only hatch in the stomachs of cows. There is no risk of invasive disease in humans from this parasite. This situation contrasts with the significant health problem of cysticercosis due to the pork tapeworm, *T. solium*, in humans.

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 shorter 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]

In cattle, immunity to reinfection develops locally in the small intestine, and is directed at newly arrived oncospheres. Challenge infection can be prevented by vaccination of cows with recombinant egg antigens.[22] Because colostrum from immune mothers protects sheep from invasion by oncospheres in experimental infections with closely related tapeworms, secretory IgA antibodies are thought to play a major role in this protective response.[23]

Figure 28.4. Gravid proglottid of *Taenia saginata*.

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 overnight, and can be discovered in bedding or clothing the following morning. A somewhat disconcerting feature is that these proglottids move in a manner very similar 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 injected with India ink, with the aid of a 26-gauge needle (Fig. 28.4). *T. saginata* proglottids have 12 or more branches on either side of the uterus.[26] In many modern labs these uterine branches are now 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. If an egg is seen on stool examination, the species cannot be determined on visual microscopy 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]

Figure 28.5. *Taenia* spp. eggs. They cannot be differentiated from eggs of other members of the taeniid family.

Adult worms remain in the intestine. Only proglottid segments pass in the stool. On the rare occasions that the scolex passes, such as after anti-parasitic therapy, it can be speciated. *T. saginata's* scolex has four lateral suckers but no hooks. The scolex of *T. solium* also has four suckers, but in addition, it has a double row of hooks.[26] Stool examination for eggs or proglottids is insensitive, since the passing of segments and eggs occurs only intermittently. Caution needs to be exercised by diagnostic parasitology technicians, because the eggs of *T. solium* are infectious for humans. An additional diagnostically relevant test is the sticky tape 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 now advanced and loop-mediated isothermal amplification (LAMP) and multiplex PCR tests have become available.[34, 35] An ELISA test has been developed that detects soluble *T. saginata* antigens in stool samples (coproantigens) of infected humans.[36]

Treatment (see Clinical Appendix)

Praziquantel (5–10 mg/kg) is effective for the treatment of *T. saginata* infection, and often allows recovery of the intact scolex, confirming cure of the patient.[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

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 through proper disposal of human feces but has proven difficult in some parts of the world, since untreated human feces is widely used as fertilizer. Proper education and adopting of composting approaches for human waste can destroy helminth eggs during the thermophilic phase and allow for the use of human waste as fertilizer while minimizing risk of tapeworm infection from this source.[41] Infection is fully prevented by cooking beef thoroughly, or by thoroughly freezing it prior to cooking. This is also not easily done, as people throughout the world enjoy eating rare or even raw beef. Meat inspection programs are effective in identifying contaminated meat, but inspection is not carried out in many 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 where vaccines are affordable.[22, 42] 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]

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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 mature 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 egg of *T. solium* can infect the human host, resulting in a condition called cysticercosis. This juvenile-stage infection can be serious, even fatal, particularly if the larval tapeworm invades the CNS.[4, 5] Neurocysticercosis ranks among the most common causes of acquired epilepsy, worldwide.[6]

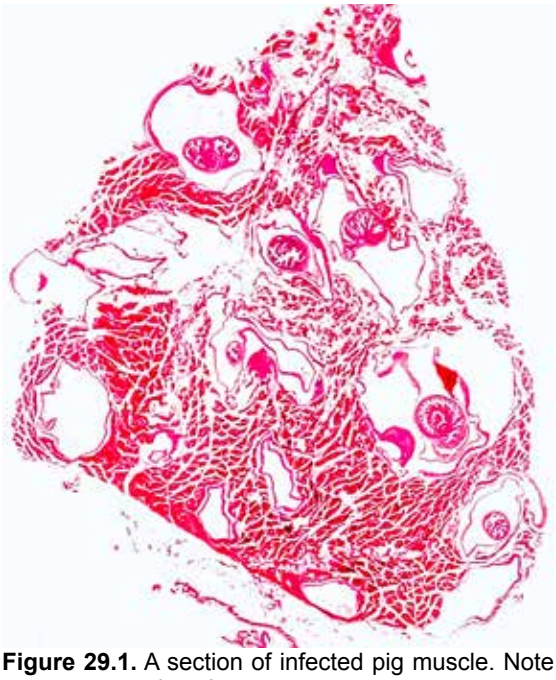


Figure 29.1. A section of infected pig muscle. Note numerous cysticerci.

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 even 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, 13] 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 in order 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

The history of the different tapeworms is initially intertwined, as it was not until many years after their initial discovery that it was determined that these similar parasites were fundamentally different in terms of their morphology and host specificity.[18] In 1683, Edward Tyson described several tapeworms, which he recovered from dogs.[19] Tyson is credited as the first person to recognize the head or scolex of these tapeworms.[18] In 1656, Félix Plater, a Swiss physician, wrote about the distinctions between *Taenia* spp. and *Diphyllobothrium latum* (then called *Lumbricus latus*).[20] In 1782, Johann Goeze described the adult tapeworm, but it was Aristotle who wrote about the cysticercus stage of the worm in the muscles of pigs.[21] There is no evidence that Goeze comprehended the relationship between the larval infection in the pig and the adult infection in humans. In 1688, Philip Hartmann described cysticercosis in pigs.[22] 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.[23, 24]

Life Cycle

The phase of the life cycle that occurs in humans is dependent 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 encapsulated cysticercus (i.e., juvenile) stage of the worm (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 with the aid of its four suckers and crown of hooklets. In an experimental infection of hamsters with oncospheres of *T. solium*, the attachment site in the gut tract was studied.[25] 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.

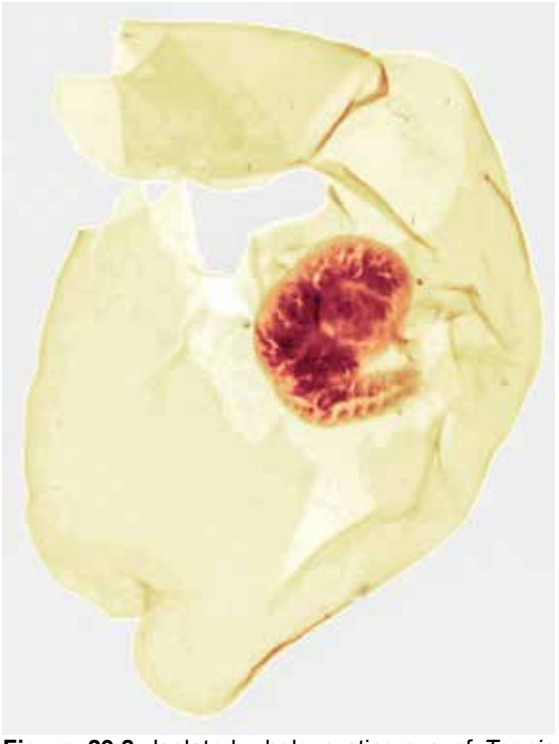


Figure 29.2. Isolated whole cysticercus of *Taenia solium*, measuring 2–5 mm in diameter.

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 a three-month period, 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 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

Figure 29.3. Whole adult *T. solium*. Arrow indicates scolex.

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.

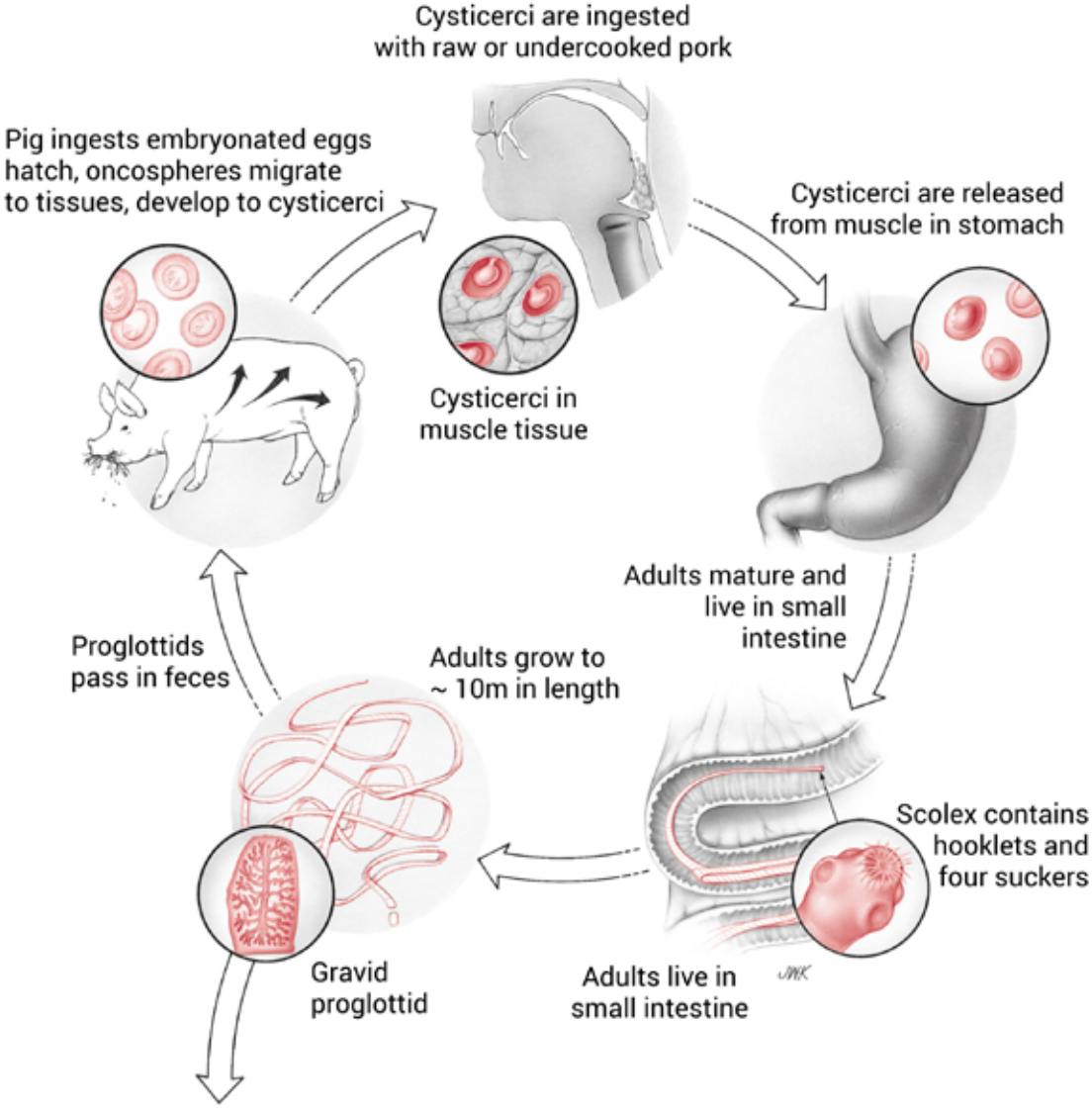
Unlike infection with *T. saginata*, the tissue phase of the life cycle can occur in humans, as well as in pigs following ingestion of embryonated *T. solium* eggs (Figs. 29.5, 29.6). When the egg is ingested, the larva (oncosphere) inside survives the gastric acid of the stomach and enters the small 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 mm^3. Cysticerci achieve maximum 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 it does elicit the formation of humoral antibodies.[26] 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.[27] A similar situation

Figure 29.4. Scolex of *Taenia solium.* Note four suckers and hooks. Photo E. Gravé.

Taenia solium



Cysticercosis (Taenia solium)

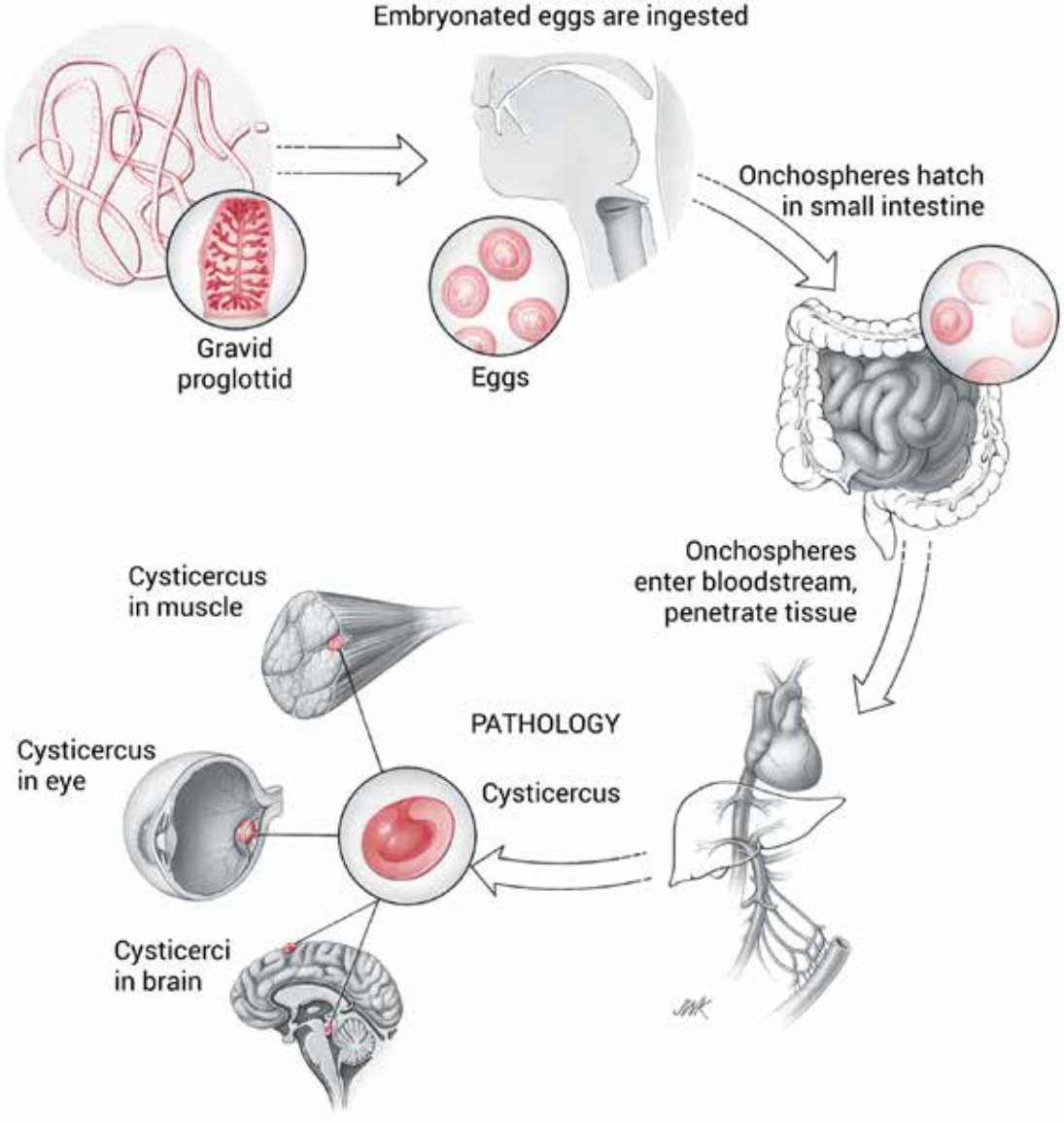


Figure 29.5. *Taenia* spp. egg. The species of tapeworm cannot be determined based on its morphology. Eggs measure 30-40 μm in diameter.

exists between the cysticercus of *T. saginata* and cattle. The cDNAs encoding two *T. solium* glucose transporter molecules have been expressed in bacteria, and their fusion proteins identified and characterized.[28] One is on the tegumental surface of the adult worm (TGTP1) and the other is on the surface of the larva (TGTP2). Both molecules have the potential for being 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.[29, 30]

In the tissue phase, much of the pathological consequences are those of a space-occupying lesion and the inflammation that results upon death of the parasites.[31] Oncospheres that lodge in the CNS (e.g., eye, and brain; Figs. 29.8, 29.9) develop with little immune response until the parasites start to die, triggering an intense immune response accompanied by edema, and in many cases, seizures.[32]

Oncospheres, other than those in the CNS, are presumably more susceptible to immune attack, but their rapid development to the cysticercus stage may account for the high rate of survival, at least during a primary infection. Cysticercus development is coincident with modulation of host immune responses, favoring expression of parasite immune evasion mechanisms that interfere with Th2-type protective immune responses.[32] Immunomodulatory substances have been identified and partially characterized; for example, Taeniastatin, a protease inhibitor, and paramyosin, which inhibit different aspects of the complement cascade.[33-35]

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 in part due to the result of interleukin signaling from T cells. Specific IgM antibodies and natural killer cells are also present in these infiltrates.[36] An acute local inflammatory reaction follows, consisting of lymphocytes, plasma cells, and eosinophils, which may be clinically significant in neurocysticercosis.[32] 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

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.

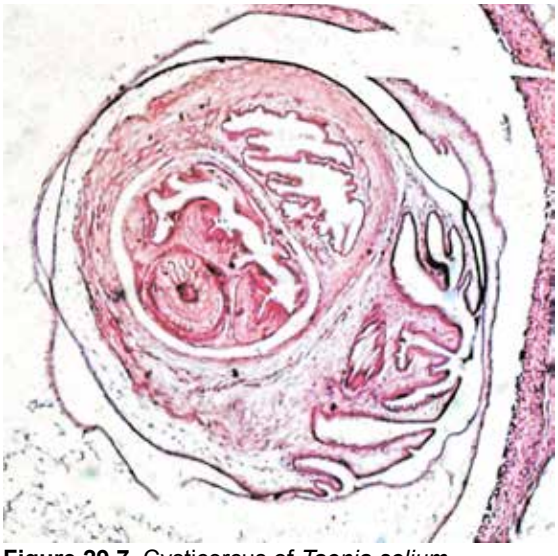


Figure 29.7. Cysticercus of *Taenia solium*.

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.[37]

Clinical Disease

There are two very distinct forms of infection with *T. solium*: intestinal infection with the adult parasite following ingestion of raw or undercooked pork and invasive disease following ingestion of embryonated eggs from a human with an intestinal infection. Intestinal infection with adult *T. solium* causes few symptoms other than perhaps epigastric fullness in some individuals. Patients are usually asymptomatic, and do not become aware of their infection until they discover the proglottids in stool, on the perianal skin, in clothing, or bed sheets. Some patients report abdominal pain, distension, diarrhea, and nausea, but there are no controlled studies to demonstrate their link with the presence of *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 antihelmintic therapy, but the connection to infection with *T. solium* is still unclear.[38]

Invasive disease 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 of the patients with neurocysticercosis also have evidence for disease in other parts of the body.[6]

Extraneural cysticercosis is usually asymptomatic, but patients may notice painless subcutaneous nodules in the arms or chest, or small, discrete swellings of particular muscles (Fig. 29.10), in which cysticerci have lodged.[39] After several months, the nodules become swollen and tender, presumably because of the host inflammatory response to the cysticerci. Subsequently, the nodules disappear. There may be geographic variation in this clinical presentation because subcutaneous cysticercosis is common in Asia

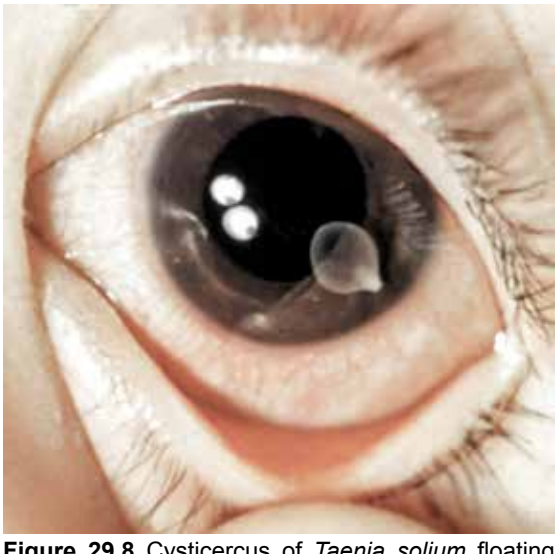


Figure 29.8 Cysticercus of *Taenia solium* floating free in the anterior chamber of the eye.

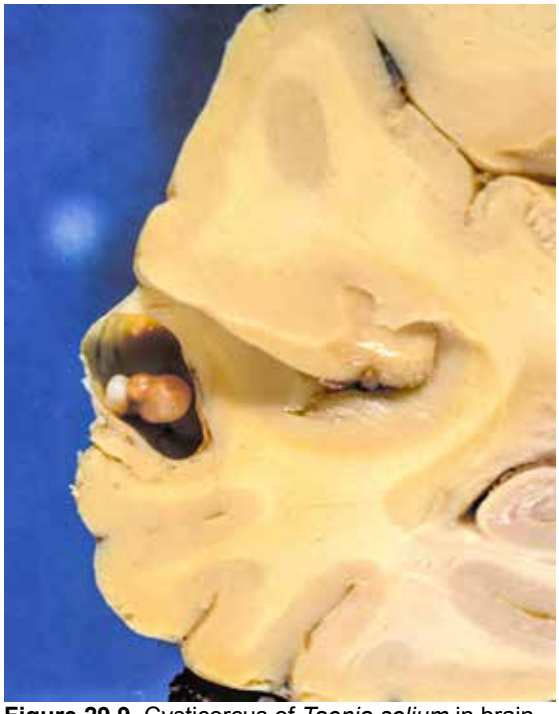


Figure 29.9. Cysticercus of *Taenia solium* in brain.

and Africa, but rare in Latin America.[6] Ultimately, these lesions will calcify after death of the parasite. The calcifications can persist for years. Some patients with extraneural cysticercosis can also harbor cardiac cysticerci, which is usually also asymptomatic.[6]

Neurocysticercosis (cysticercosis in the CNS) is the most severe manifestation that follows the ingestion of *T. solium* eggs. The signs and symptoms vary greatly with the number and distribution of cysts (Figs. 29.8 – 29.13).[39] It often presents as a space-occupying lesion, and in many respects mimics a tumor.[31] Seizures, hydrocephalus, and focal neurologic abnormalities may be the presenting signs.[40] It has been estimated that seizures occur in 50–80% of patients with brain parenchymal cysts, which occur as a consequence of host inflammation around dead and degenerating parasites.[6, 41] In endemic regions, teenage and young adults exhibit the highest rates 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.[42] Usually, with those suffering seizures, the cysticercus has begun to degenerate and in some cases started to calcify (Fig. 29.13).[43]

People still residing in endemic areas are often found to have more complex disease, and have 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.[44] 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

Figure 29.10. Radiogram of lower leg with numerous cysticerci of *Taenia solium*.

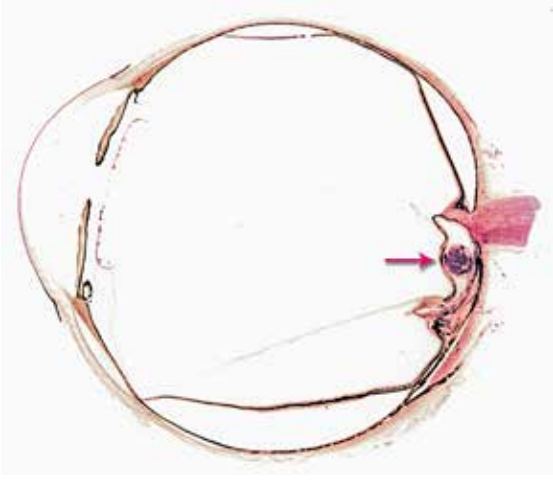


Figure 29.11. Cross section of whole eye with cysticercus of *T. solium* (arrow) in retina at the level of the optic nerve.

with a single enhancing brain lesion, whereas in Latin America it is common to find multiple cysts without evidence of inflammation.[6]

A less common but challenging disease clinically is extraparenchymal neurocysticercosis with disease in the ventricles, subarachnoid space, and the eye. These infections generally increase the risk of morbidity and mortality, in large part through the elevation of 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 microscopy, by analysis of the gravid proglottids, or by recovery and identification of the scolex in feces. Overall, the sensitivity of stool microscopy is poor, and even then, *T. solium* eggs are morphologically indistinguishable from *T. saginata* eggs. Upon acid-fast staining, occasionally the species can be distinguished, as fully-mature eggs of *T. saginata* have an acid-fast shell and eggs of *T. solium* are not acid-fast.[45, 46] A DNA dot blot test can differentiate *T. solium* from *T. saginata*.[47] NAAT as well as fecal antigen tests are now available for diagnosis and differentiation of *T. solium* eggs from *T. saginata*.[48-50] Perianal scraping with adhesive tape to trap eggs has been used to improve the sensitivity of microscopy, but this technique is not as effective for *T. solium* infection as it is for *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). These proglottid segments can also be prepared and viewed using standard hematoxy-

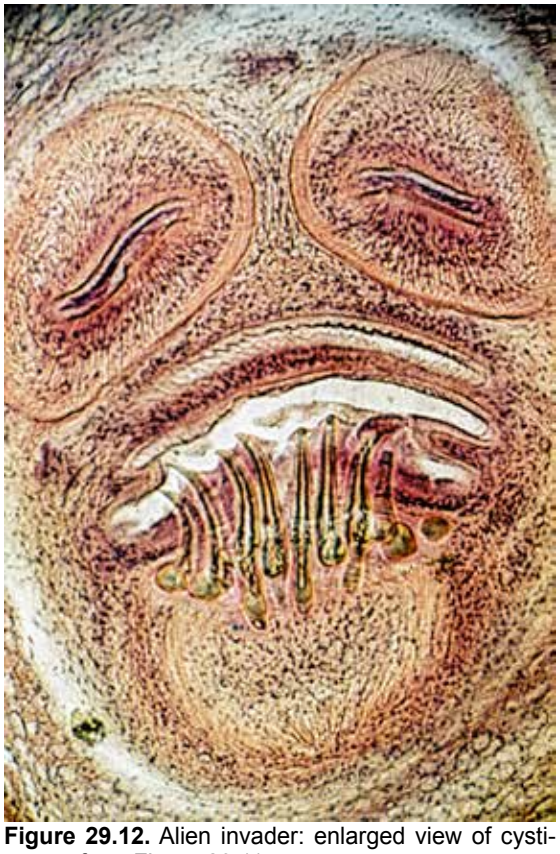


Figure 29.12. Alien invader: enlarged view of cysticercus from Figure 29.11.

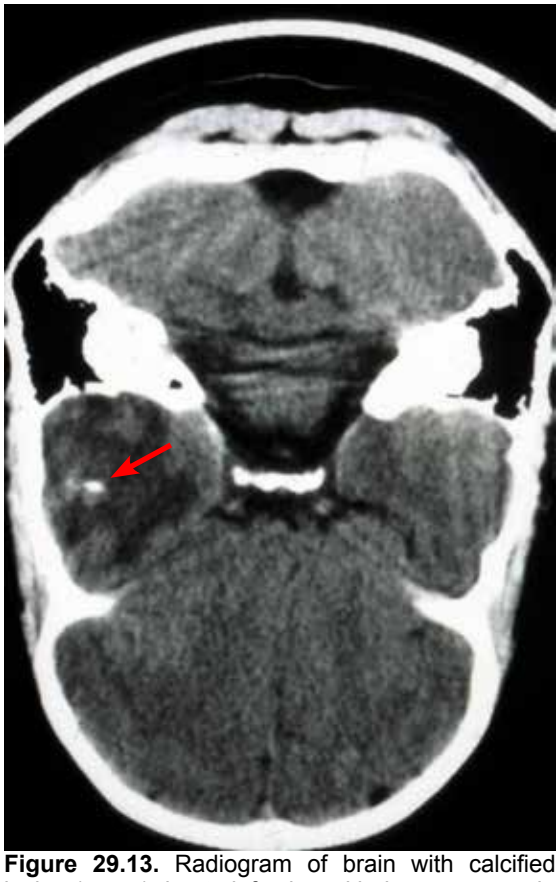


Figure 29.13. Radiogram of brain with calcified lesion (arrow) due to infection with the metacestode of *Taenia solium*.

lin and eosin staining. Extreme caution must be exercised when handling unfixed proglottids of *T. solium*, since the embryonated eggs are infectious.

Because of the distinct morphological features of the *T. solium* scolex compared with that of *T. saginata*, the gold standard of taeniasis diagnosis had traditionally been the recovery and visualization of this portion of the parasite. Polyethylene glycol salt purges to improve bowel cleaning significantly improves the likelihood of scolex recovery, but is not often used.[51]

ELISA performed on fecal extracts for antigen detection is sensitive (95%) and specific (99%).[52] Patients with adult tapeworms also produce antibodies detectable by immunoblot assay.[53] Serological assay is particularly useful for epidemiological studies.

Diagnosis of cysticercosis is usually based on imaging tests and criteria have been established.[6, 44, 54] Neuroimaging by CT or MRI is of critical importance in diagnosing 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 that represents 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 con-

Figure 29.14. Gravid proglottid of *Taenia solium*. Uterus contains less than 12 lateral branches on each side.

firmatory 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, 55-57] 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.[58]

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*, but its use must be tempered by the possibility that this treatment will also destroy occult cysticerci in the brain, and trigger CNS manifestations.[6] If neurocysticercosis has either been effectively ruled out or treated, then treatment for the intestinal worms can be given. 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 leading to cysticercosis.[59, 60] Niclosamide is an alternative for adult *T. solium* when praziquantel is unavailable (2 g orally in a single dose).[6, 61]

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).[62] 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.[63]

Albendazole has become the drug of choice for neurocysticercosis with better efficacy than praziquantel and fewer drug interactions with steroids and seizure medications.[64, 65] Often albendazole is combined with praziquantel due to evidence demonstrating improved effect with combination therapy, but in this context interactions with corticosteroids and seizures medications need be managed.[66] It is the inflammatory response in the brain that is largely responsible for seizures, encephalitis, and elevated intracranial pressure. 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 was 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, 66] All patients selected for treatment

with antihelmintic drugs should undergo a prior ophthalmologic exam in order to rule out intraocular cysts and be counseled about the risk of treatment-induced seizures.

Guidelines on the use of antihelmintic chemotherapy have been published.[67] Since patients with single ring-enhancing lesions on CT scans often improve spontaneously without any therapy, they can be followed and not given albendazole or steroids.[68, 69] In the case of multiple viable cysticerci, most clinicians suggest treatment with albendazole and prednisone, with monitoring for elevations in intracranial pressure and seizures.[4, 6]

Because both albendazole and praziquantel kill cysticerci and exacerbate brain inflammation and edema, 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.[70] Additional medical management with anticonvulsants is also a critical component of the long-term management of *T. solium*-induced seizures.

Neurosurgical management with placement of ventricular shunts is often required for complicated neurocysticercosis involving cerebrospinal obstruction and hydrocephalus. Surgical management may also be required for spinal lesions and eye lesions.

Clinical practice guidelines that provide treatment recommendations were updated in 2018.[58]

Prevention and Control

Taenia solium is a significant public health problem, even outside the endemic areas, due to the association of cysticercosis with the adult infections.[71] For example, an outbreak of cysticercosis was reported among an orthodox Jewish community in New York City resulting from the ingestion of *T. solium* eggs passed from domestic employees who were recent emigrants from Latin America.[72] 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.[72]

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 infection in pigs 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.[73]

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 interventions comprised of sanitation and pig management prove highly effective in disease control.[74] Ring-screening strategies have been proposed to control *T. solium* infection.[75] Antihelmintic chemotherapy programs need to 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]

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, where public health programs can be integrated with prevention on the farm.[37, 76-78] In one experimental study, treating all pigs eliminated 100% of viable cysticerci.[79] Serological testing in the abattoir is now also possible.[80] 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, most likely as the result of feeding at garbage dumps near campgrounds.[81] Hunting bears is a popular sport in the United States, and distributing meat from kills to neighbors is common practice among hunters. Hunter organizations should issue warnings, advising hunters that any meat obtained from carnivores or omnivores should be cooked well before eating.

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30. *Diphyllobothrium latum*
(Linnaeus 1758)

Pronunciation: \dī-fil-ə-bäth-rē-əm\\lät-əm\

Introduction

Diphyllobothrium latum (\DYE-fil-ow-both-REE-um\\la-tum\) belongs to the order Pseudophyllidea and usually grows to a length of 2–15 meters. It has achieved a recorded length of 25 meters, making it the longest parasite to infect humans.[1] It is estimated that 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-ˈdri-tik-əm\), *D. lanceolatum* (\LAN-see-o-LATE-um\, \ˈlan-sē-ə-ˌlāt-əm\), *D. dalliae* (\DAL-ee-uh\), and *D. yonagoensis* (\Yon-uh-go-en-sus\).[2, 3] The fish tapeworm can grow at a rate of 22 cm/day, releasing 1,000,000 eggs per day, and live for up to 25 years.[4-6] It is acquired by eating raw or under-cooked fish and for that reason it is commonly referred to as the fish tapeworm. With its proglottids being wider than long it is also commonly known as the broad tapeworm. As with all other adult tapeworms, it

Figure 30.2. The scolex and mature proglottids of *D. latum.* Courtesy H. Zaiman.

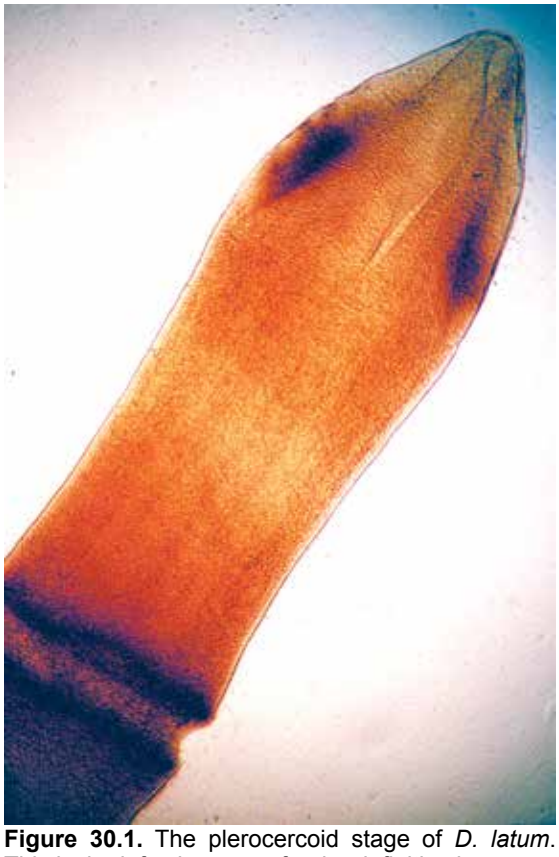

Figure 30.1. The plerocercoid stage of *D. latum*. This is the infective stage for the definitive host.

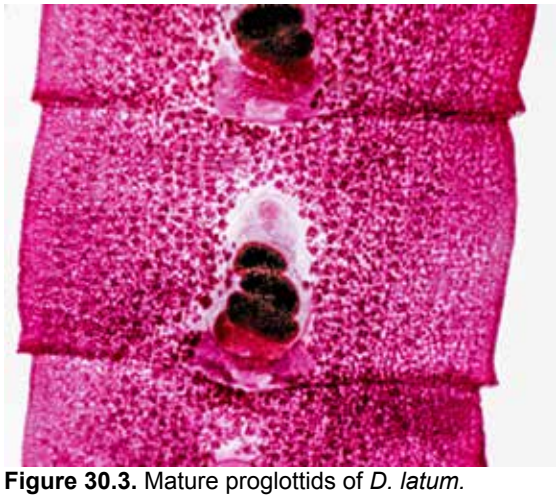

Figure 30.3. Mature proglottids of *D. latum.*

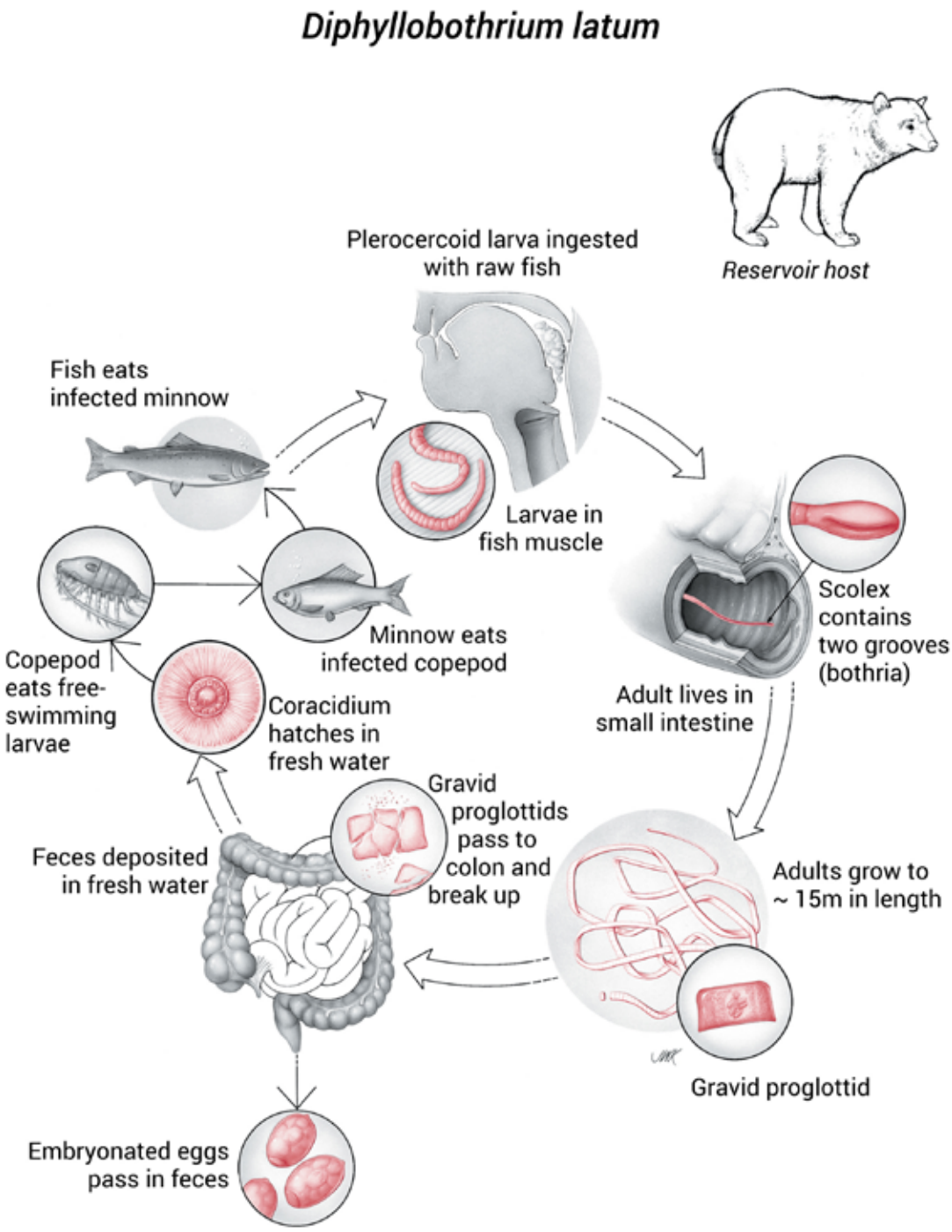
Diphyllobothrium latum
Reservoir host
Plerocercoid larva ingested with raw fish
Fish eats infected minnow
Larvae in fish muscle
Scolex contains two grooves (bothria)
Adult lives in small intestine
Minnow eats infected copepod
Copepod eats free-swimming larvae
Coracidium hatches in fresh water
Gravid proglottids pass to colon and break up
Feces deposited in fresh water
Adults grow to ~ 15m in length
Gravid proglottid
Embryonated eggs pass in feces

Figure 30.4. Gravid proglottids of *D. latum*.

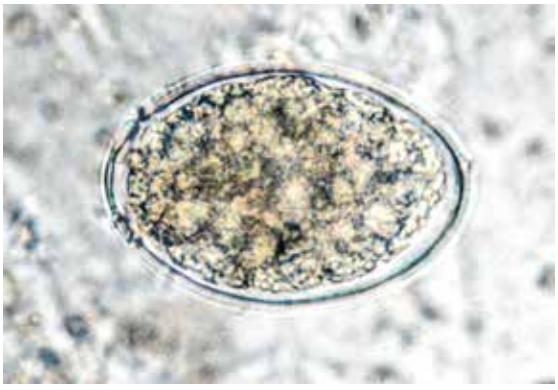

Figure 30.5. Unembryonated egg of *D. latum*. 65 μm x 45 μm.

Figure 30.6. Hatching egg of *D. latum*. Note operculum.

lives in the lumen of the small intestine and usually does little harm to its host. It has a 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 of tapeworms have similar complex life cycles. In most cases they use invertebrates (e.g., copepods) and freshwater fish as intermediate hosts. Most

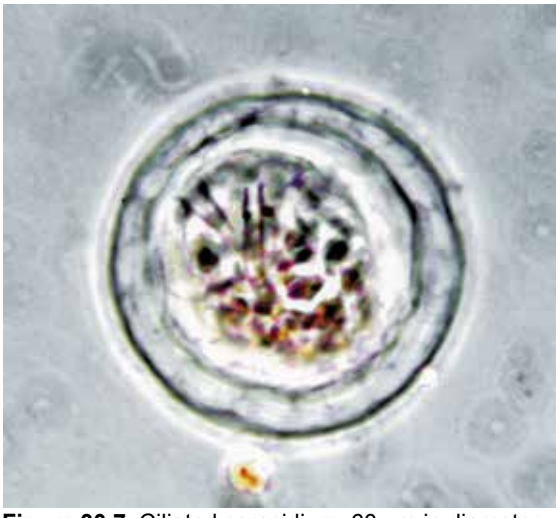

Figure 30.7. Ciliated coracidium. 60 μm in diameter.

carnivores are susceptible to infection with *D. latum*, including dog, bear, cat, fox, martin, mink, and other wild mammals. Some of these hosts are important reservoirs for the human infection.

D. latum, the major fish tapeworm of humans, will be the model in this chapter for all the others. It is still common throughout Scandinavia, though prevalence in that region has decreased due, in large part, to vastly improved sanitation.[7, 8] The infection was probably introduced to North America (especially in the Great Lakes region and in 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]

Overall, it is believed that the number of new cases of *D. latum* infection is declining in North America but human *D. nihonkaiense* infection has been reported from Washington State in the United States.[10, 11] A related subspecies, *D. latum* var. *parvum* infects humans in Korea and China.[12] In circumpolar regions, infection due to *D. dendriticum* (a more benign variant) is common in both people and animals.[13] *Diphyllobothrium pacificum*

(also known as *Adenocephalus pacificus* (\a-deno-SEF-a-lus\,\ə-ˈde-nə-ˈsefələs\)), has been reported from the Pacific Coast of South America - Peru, Chile, and Ecuador as well as Australia – and may be an important species in these regions (possibly linked to ceviche consumption in some situations).[14, 15]

Historical Information

Descriptions of what appears to have been infection due to the broad or fish tapeworm 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 experiments, described and illustrated its complex life cycle, including copepods, fish, and then the human host.[19]

Life Cycle

Infection begins when undercooked or raw fish, infected with the plerocercoid metacestode 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 the adult worms (Fig. 30.2), becoming fully formed strobilae within three months. In human infection, eggs begin to pass into the stool 15–45 days after ingestion of 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]

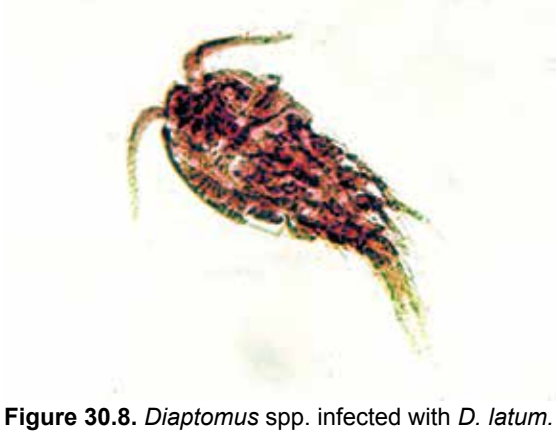

Figure 30.8. *Diaptomus* spp. infected with *D. latum.*

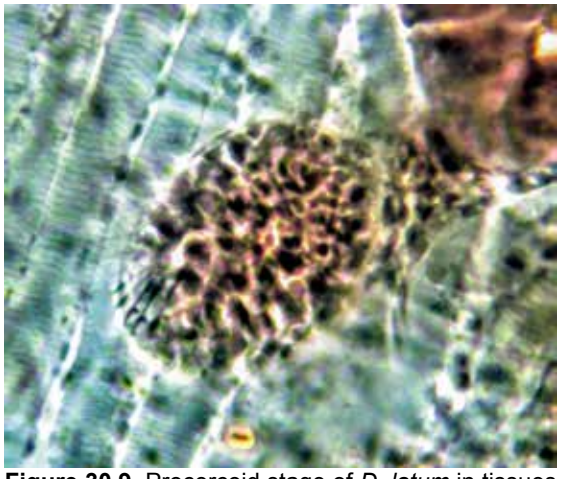

Figure 30.9. Procercoid stage of *D. latum* in tissues of *Diaptomus* spp. Note hooklets. (Phase-contrast).

Proglottids (segments) are greater in width than length (Figs. 30.3, 30.4), and contain both sets of sex organs. Proglottids fertilize eggs in nearby segments. As fertilized proglottids become gravid, eggs (Fig. 30.5) exit from the centrally located uterine pore, entering into 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 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 intermediate host, the coracidium burrows into the body cavity and develops into an immature metacestode, referred to as a procercoid (Fig. 30.9).

When infected crustaceans are consumed by small freshwater fish, particularly various species of minnows, 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. Upon death of this second intermediate host, the plerocercoids in viscera are triggered 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 analogues 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 the production of propionate and these two enzymes are integral to that metabolic pathway.[21] These tapeworms have the ability to absorb B12 at an absorption rate of 100:1 relative to that of 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. It appears that host factors, the number of infecting worms, and the specific tapeworm involved determines 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* is not well understood. There may be host genetic factors that predispose certain infected individuals to suffer the effects of this deficiency. One study indicated that patients with megaloblastic anemia due to infection with *D. latum* had less intrinsic factor than those who were free of anemia, but who also harbored the worm. This observation has not been confirmed by other studies.[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 to the fact 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 that also allow 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 potentially sustain the life cycle. Interference with this phase of its ecology would be very difficult, if not impossible. 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 greatly reduced prevalence of *D. latum*. In Scandinavian countries, gravlax and a wide variety of other marinated raw fish dishes, remain sources of infection for this tapeworm.

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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-ˈnäl-ə-pəs\\na-nə\

Introduction

Hymenolepis nana (\HI-men-OL-eh-pis\\ Na-nuh), in the order Cyclophyllidea, has a worldwide distribution, and infects mostly children, with prevalence in children as high as 25% in certain areas.[1-3] In Asia and elsewhere, *H. nana* infection is a common infection among children living in poor neighborhoods and in 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 hooks. Rodents are significant reservoir hosts for this tapeworm. Like *Strongyloides stercoralis*, *H. nana* is able to 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]

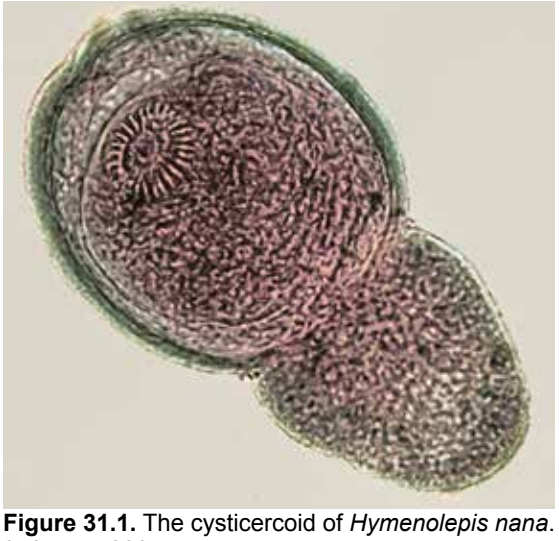


Figure 31.1. The cysticercoid of *Hymenolepis nana*. 350 μm x 200 μm.

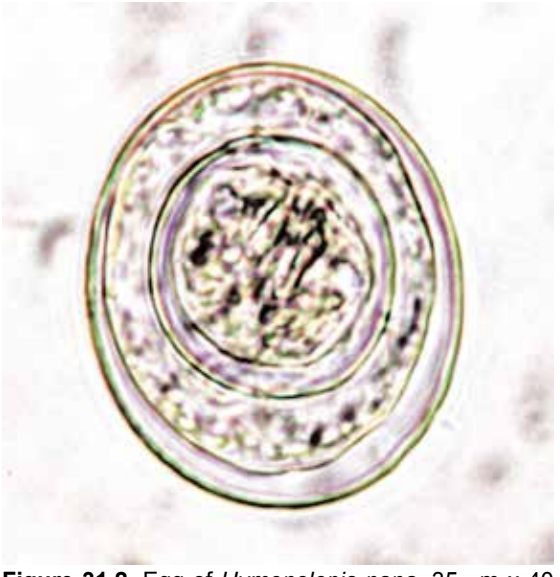


Figure 31.2. Egg of *Hymenolepis nana*. 35 μm x 40 μm.

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.

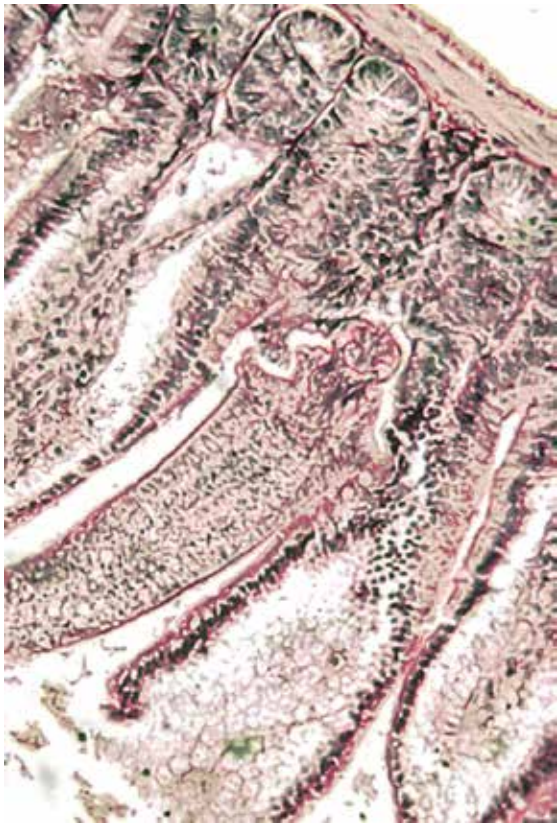


Figure 31.3. Histological section of *H. nana*, *in situ*.

31.1) along with an infected insect, or by ingesting embryonated eggs. Infective stages 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 nearby 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 a possibility, 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, or by rodents, or by humans. In the invertebrate host, the oncospheres hatch and penetrate the gut and enter the hemocele 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 development of protective immunity and is most likely a consequence of children ingest-

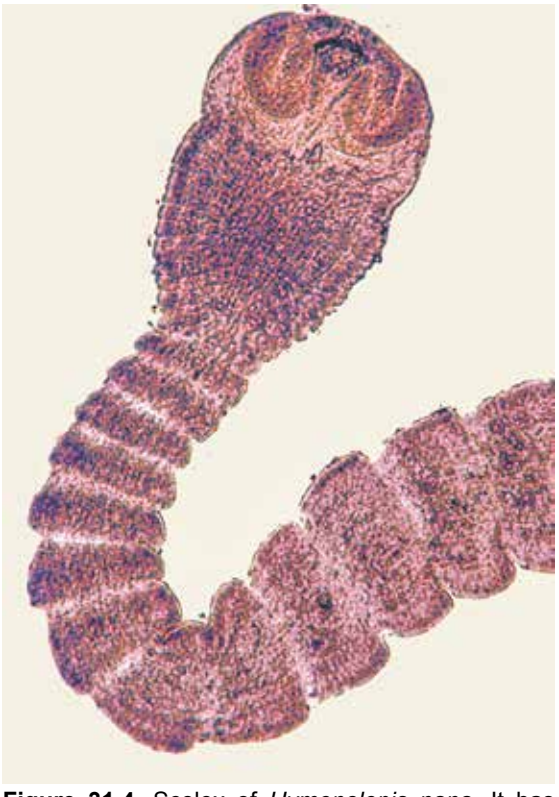


Figure 31.4. Scolex of *Hymenolepis nana.* It has four suckers as well as hooks.

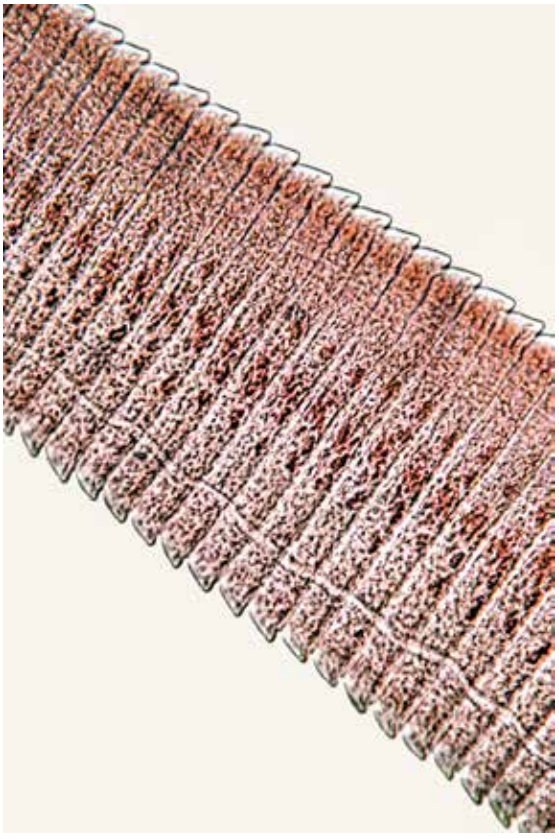


Figure 31.5. Mature proglottids of *Hymenolepis nana.* 400 μm wide.

ing infected insects. The cysticercoid stage is relatively non-immunogenic, allowing for autoinfection to develop. In contrast, infection initiated by ingestion of the egg stage triggers a rapid and robust protective immune response.[15, 16] A low, but detectable, protective humoral immune response occurs as the result of 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 establishment of new infections with *H. nana*.[18, 19] In addition, 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] It appears that immunity to this parasite is multifactorial, involving both Th1 and Th2 responses.[17]

Clinical Disease

Most infections are not clinically apparent. Heavy infections are accompanied by diarrhea.[5] It is not clear 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 not effective 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 environment and, in many situations, controlling their populations has reduced the incidence of infection. However, maintaining small rodent populations is challenging. Reinfection in endemic areas is the norm.

Hymenolepis diminuta

(Rudolphi 1819)

Pronunciation: \hī-mə-ˈnäl-ə-pəs\\də-mi-nü-tə\

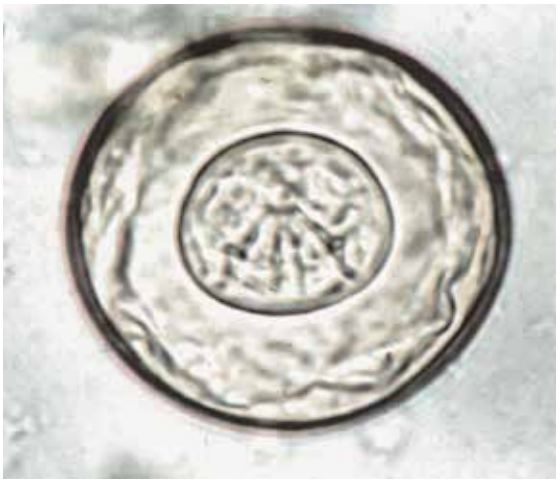


Figure 31.6. Egg of *H. diminuta*. 75 μm in diameter.

Introduction

Hymenolepis diminuta (\HI-men-OL-oh-pis\ di-min-oo-tuh) is found throughout the world 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

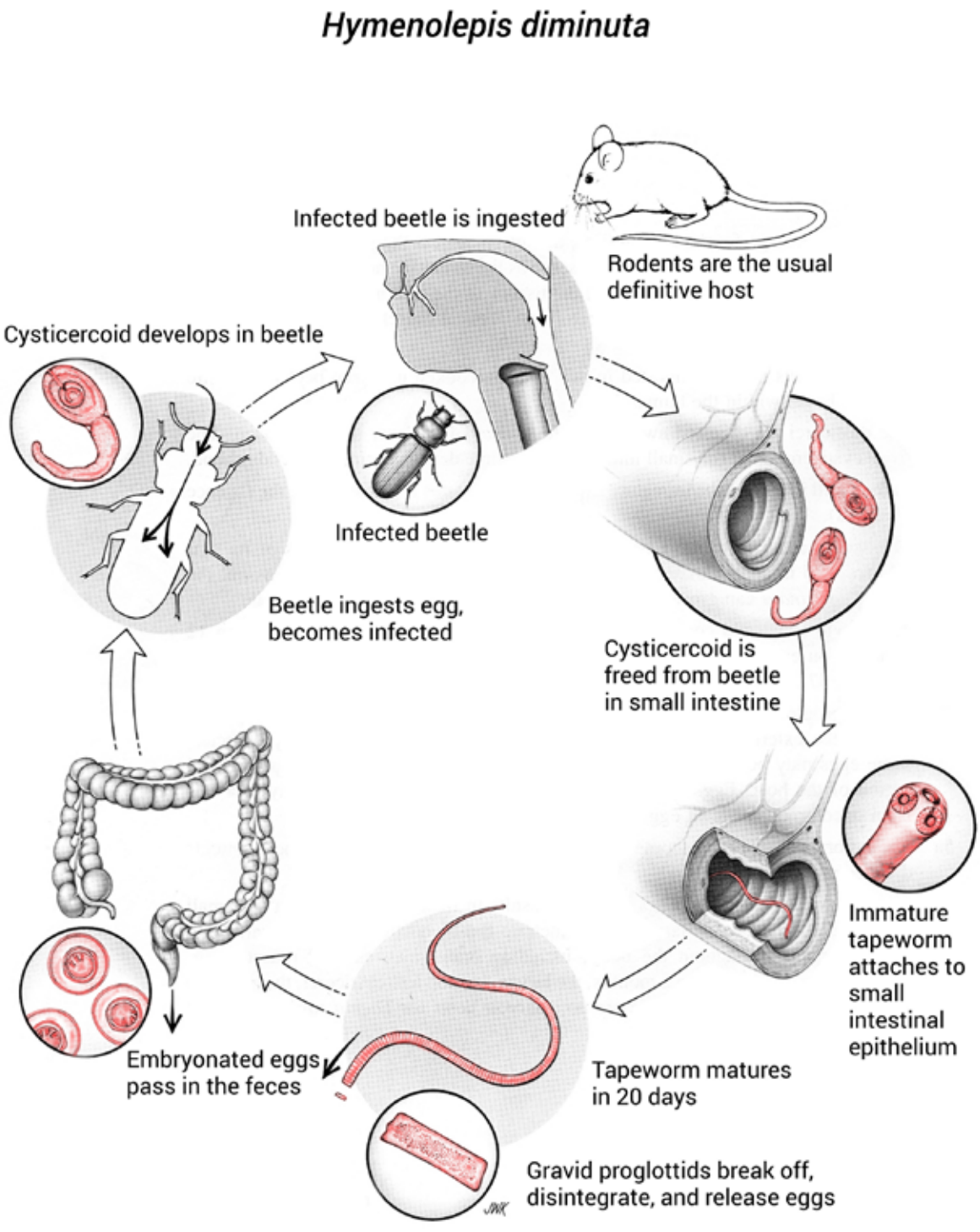
Infection begins when the cysticercoid is ingested with the infected insect. The immature worm attaches to the intestinal wall with the aid of four suckers on its scolex. The adult worm matures within 18 days and grows to 50 cm in length. The strobila contains about 1,000 proglottids at any one time.

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 molitor* (mealworm) larvae, some eggs passed through their gut tract, and were incorporated within the fecal pellets. There, they remained infective for 48 hours, allowing infection to spread 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, and 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
Infected beetle is ingested
Rodents are the usual definitive host
Cysticercoid develops in beetle
Infected beetle
Beetle ingests egg, becomes infected
Cysticercoid is freed from beetle in small intestine
Immature tapeworm attaches to small intestinal epithelium
Tapeworm matures in 20 days
Gravid proglottids break off, disintegrate, and release eggs
Embryonated eggs pass in the feces

Clinical Disease

H. diminuta appears to induce no tissue damage. Usually there are no clinical symptoms 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, but the latter is an unrealistic goal in most rural and suburban situations, particularly in less developed countries. Community efforts are aimed at curtailing contamination of food, especially whole grains and processed flour, by insects that could harbor the intermediate stage of the worm.

Dipylidium caninum

(Linnaeus 1758)

Pronunciation: \dī-ˌpī-ˈlid-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 the dog, cat, fox, hyena, and occasionally human. 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 cysticercoid is released from the flea by the digestive enzymes of the host. 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 eggs. As with *H. diminuta*, the oncosphere penetrates the hemocele of the immature

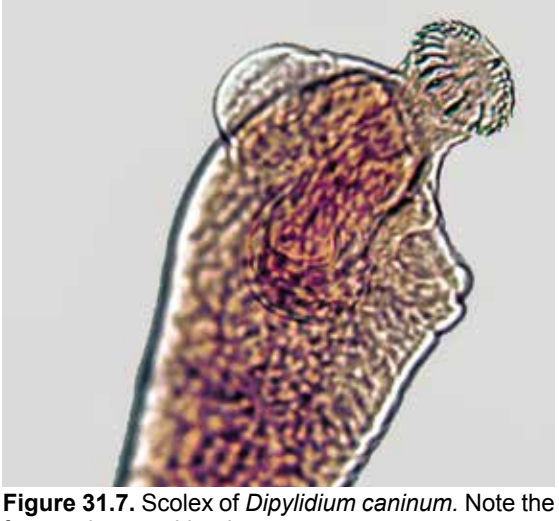


Figure 31.7. Scolex of *Dipylidium caninum.* Note the four suckers and hooks.

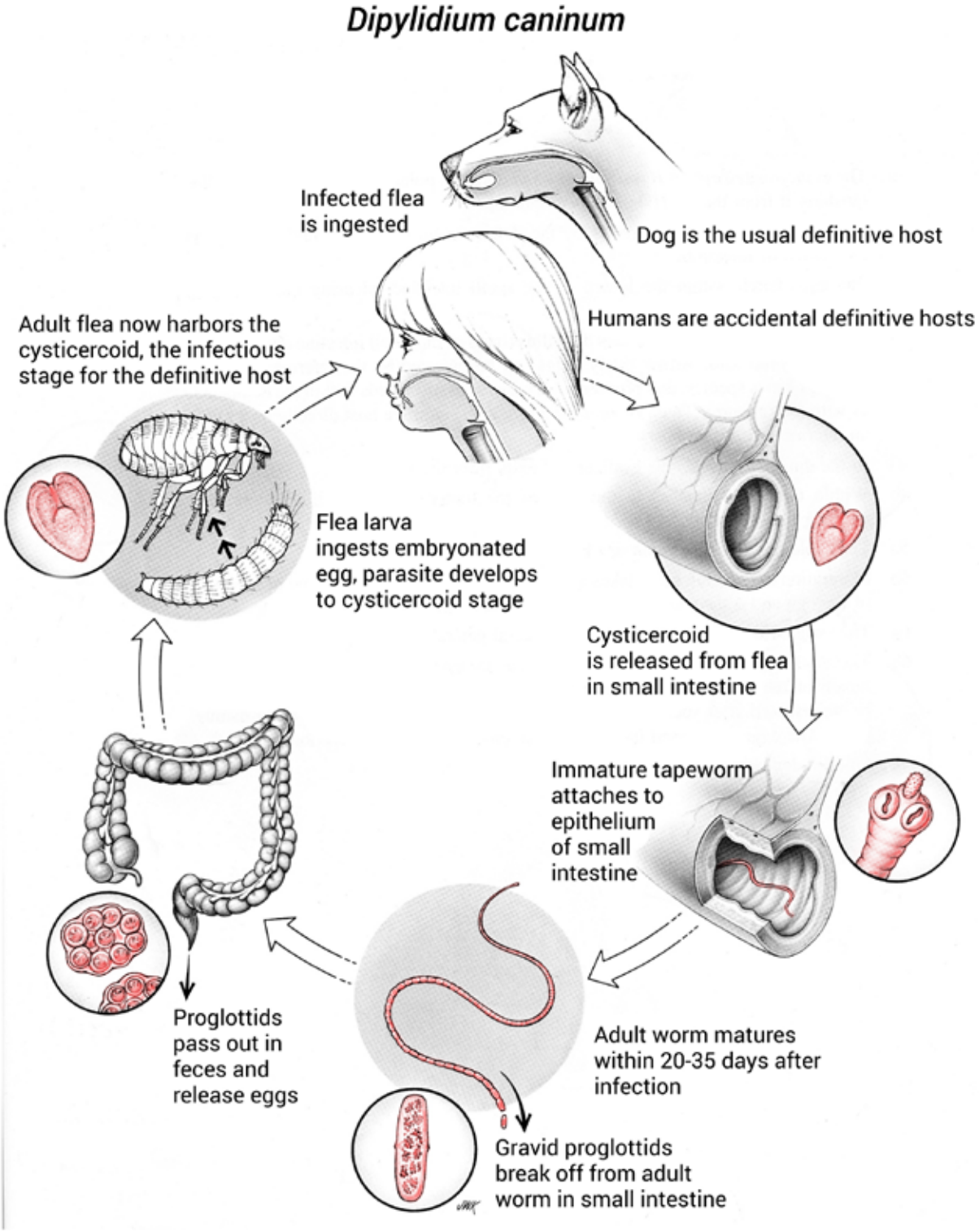

Dipylidium caninum
Infected flea is ingested
Dog is the usual definitive host
Humans are accidental definitive hosts
Adult flea now harbors the cysticercoid, the infectious stage for the definitive host
Flea larva ingests embryonated egg, parasite develops to cysticercoid stage
Cysticercoid is released from flea in small intestine
Immature tapeworm attaches to epithelium of small intestine
Adult worm matures within 20-35 days after infection
Gravid proglottids break off from adult worm in small intestine
Proglottids pass out in feces and release eggs

Figure 31.8. Double-pored gravid proglottid of *Dipylidium caninum*. 200 μm in width.

Figure 31.9. Egg cluster of *Dipylidium caninum*.

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 a number of symptoms, such as mild abdominal pain, diarrhea, pruritus ani, failure to thrive, and irritability, may be association 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 greatly reduce the chances of human infection.

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32. Juvenile Tapeworm Infections of Humans

Echinococcus granulosus
(Batsch 1786)

Pronunciation: \i-ˌkī-nə-ˈkä-kəs\ \gran-yə-lō-sis\

Echinococcus multilocularis
(Leuckart 1863)

Pronunciation: \i-ˌkī-nə-ˈkä-kəs\ \mul-tī-läk-yü-ler-is\

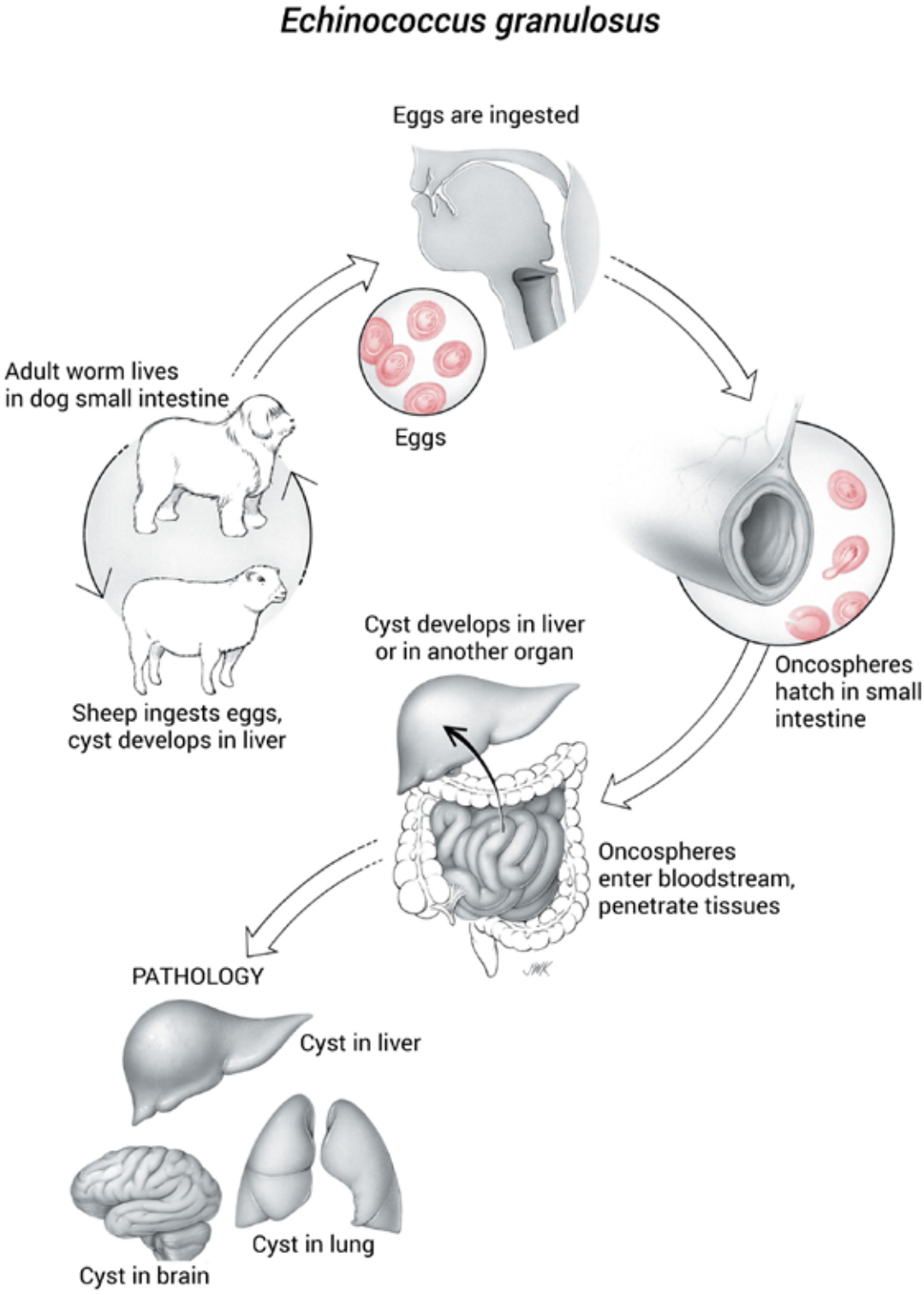
Introduction

Echinococcus granulosus (\eh-KYE-no-KOK-us\\GRAN-you-low-sis\) 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 to 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-lok-you-lair-is\) cause infection in humans, 95% of the cases of human disease are due to *E. granulosus*.[1] It is estimated that an annual loss of U.S. $194,000,000 or 285,000 disability-adjusted life years is due to echinococcosis worldwide.[2]

Figure 32.1. Adult of *Echinococcus granulosus*. Note suckers and hooks on scolex. The worm is 5.5 mm long.

Distribution of *E. granulosus* coincides with sheep husbandry. Eurasia, especially the Russian Federation and Central Asia and China (including Tibet), Mediterranean countries (especially, Turkey, Lebanon and Syria), North and East Africa (especially, Egypt, Sudan, and Kenya), and Australia have the highest prevalence.[3-5] Mongolia is also an important source of hydatid disease.[6] Infection has been totally 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]


Echinococcus granulosus
Eggs are ingested
Eggs
Adult worm lives in dog small intestine
Sheep ingests eggs, cyst develops in liver
Oncospheres hatch in small intestine
Cyst develops in liver or in another organ
Oncospheres enter bloodstream, penetrate tissues
PATHOLOGY
Cyst in liver
Cyst in lung
Cyst in brain

Indigenous peoples of the Canadian Arctic, especially the Inuit, are infected with a northern variant, *E. granulosus var. canadensis*, 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 as well as 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 greater 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, as well as 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 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. A canine host must ingest the hydatid cyst and its contents (i.e., the protoscolices) to complete the life cycle. 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, but 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 suppress 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]

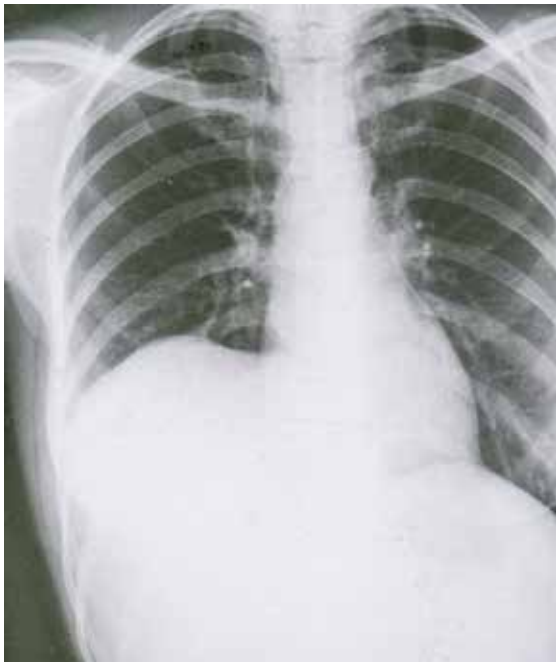


Figure 32.2. Radiogram of upper body showing elevation in right lobe of liver due to a large hydatid cyst.

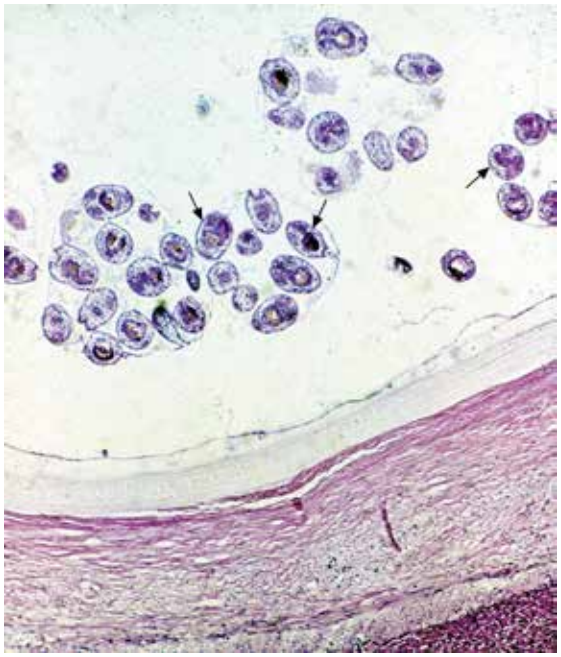


Figure 32.3. Histological section of an hydatid cyst with capsules filled with protoscolices (arrows) of *Echinococcus granulosus*. Each protoscolex measures approx. 13-14 μm in length.

Clinical Disease

The primary exposure and initial infection are thought to always be asymptomatic with symptoms only occurring coincident with cyst development. Hydatid disease develops as the result of 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

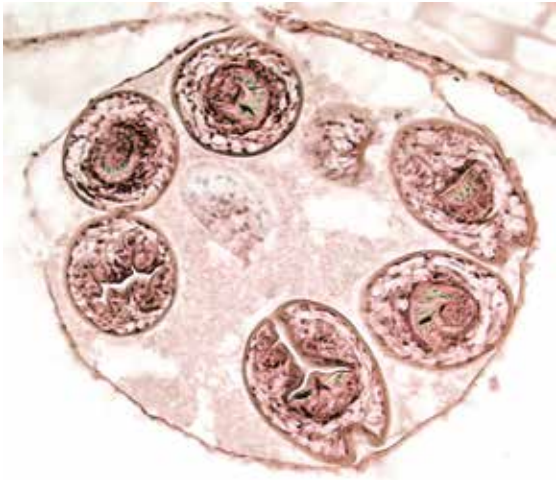


Figure 32.4. Brood capsule with protoscolices of *Echinococcus granulosus*.

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 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

Figure 32.5. Protoscolex of *E. granulosus*.

Figure 32.6. Petri dish filled with daughter cysts of *Echinococcus granulosus*.

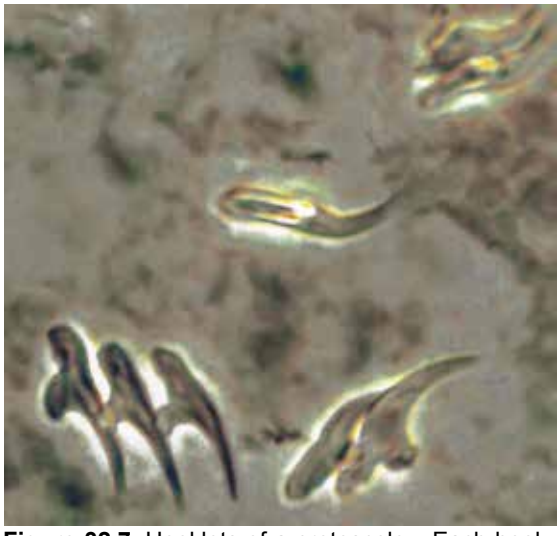


Figure 32.7. Hooklets of a protoscolex. Each hooklet measures approx. 2 μm in length.

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 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

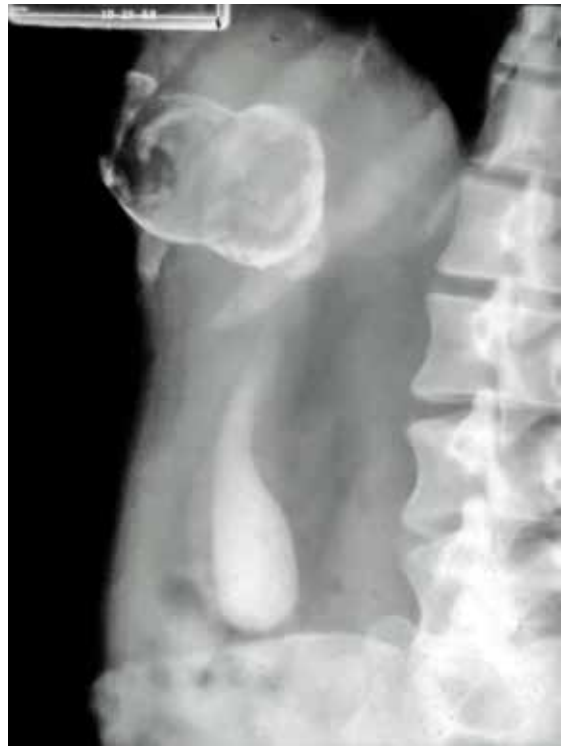


Figure 32.8. Radiogram of a calcified hydatid cyst of *Echinococcus granulosus*.

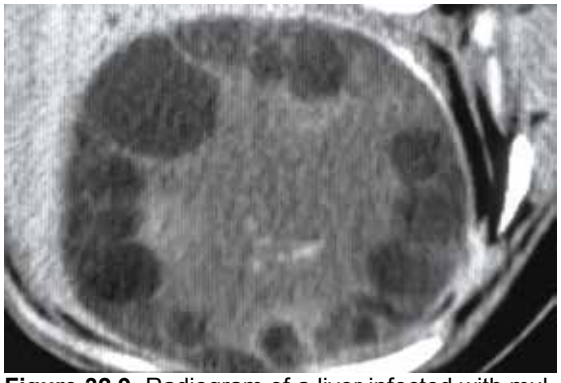


Figure 32.9. Radiogram of a liver infected with multiple hydatid cysts of *Echinococcus granulosus*.

detect circulating echinococcus antigens. Eosinophilia is an uncommon finding in patients with hydatid disease.[46]

A definitive diagnosis of echinococcosis 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 are the hooklets and portions of the acellular, laminate outer membrane of the cyst wall. Biopsy of cysts is absolutely contra-indicated, due to their metastatic nature.

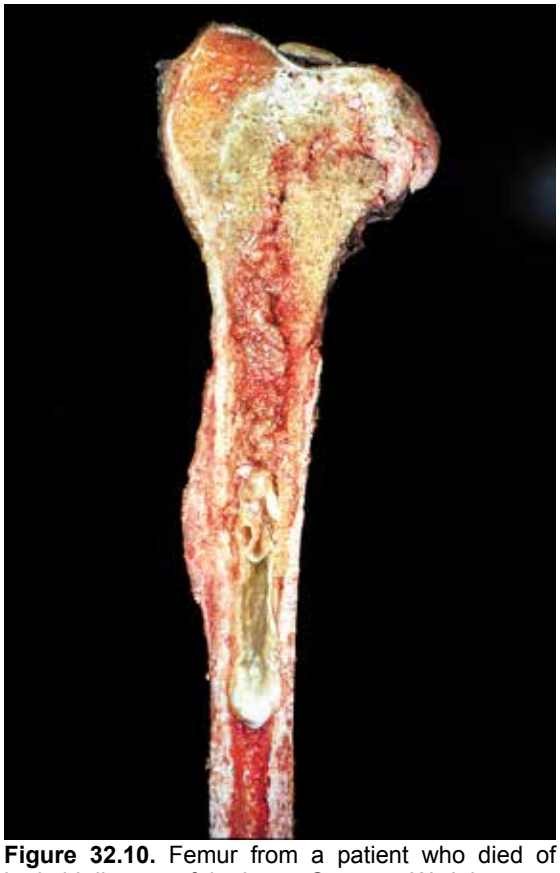


Figure 32.10. Femur from a patient who died of hydatid disease of the bone. Courtesy W. Johnson.

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.

Treatment (see Clinical Appendix)

Treatment of hydatid disease is driven by cyst size, location, and stage.[47] 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 as well as parasite factors.[48, 49] These hydatid cysts develop, enlarge, fill with protoscolices and daughter cysts and undergo a defined evolution over years with predictable features.[47] As the hydatid cyst develops, it goes through a series of defined

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.

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 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, a variety of strategies have been devised to prevent or minimize spillage of cyst contents. This includes preoperative use of antihelmintic drugs and the use of 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.[50, 51] The risk of complications from PAIR appear to be greatly reduced through the use of adjuvant antihelmintic chemotherapy started one month prior to performing this procedure. More recently laparoscopic surgery has emerged as a viable alternative for surgical treatment.[52] For pulmonary cysts antihelmintic therapy is usually started after cyst removal with concerns that preoperative antihelmintic 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 courses of therapy are usually required.[46, 50, 51] 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%.[51] Albendazole is preferable to mebendazole because the former is metabolized to a sulfoxide derivative, which exhibits 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 praziqu-

Figure 32.13. *Echinococcus multilocularis* alveolar cyst from an infected vole.

antel's protoscolicidal activity.[53-56] Therapeutic responses can be monitored radiographically and serologically. Reversible liver toxicity has been reported with prolonged therapy with albendazole.[57, 58] As outlined in the section on trichuriasis, the benzimidazole antihelminthics, including albendazole, are teratogenic and embryotoxic in laboratory animals. 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.[51] An arecoline control program in dogs has resulted in the near-elimination of *E. granulosus* infection in New Zealand and Tasmania. In Iceland, 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.[59]

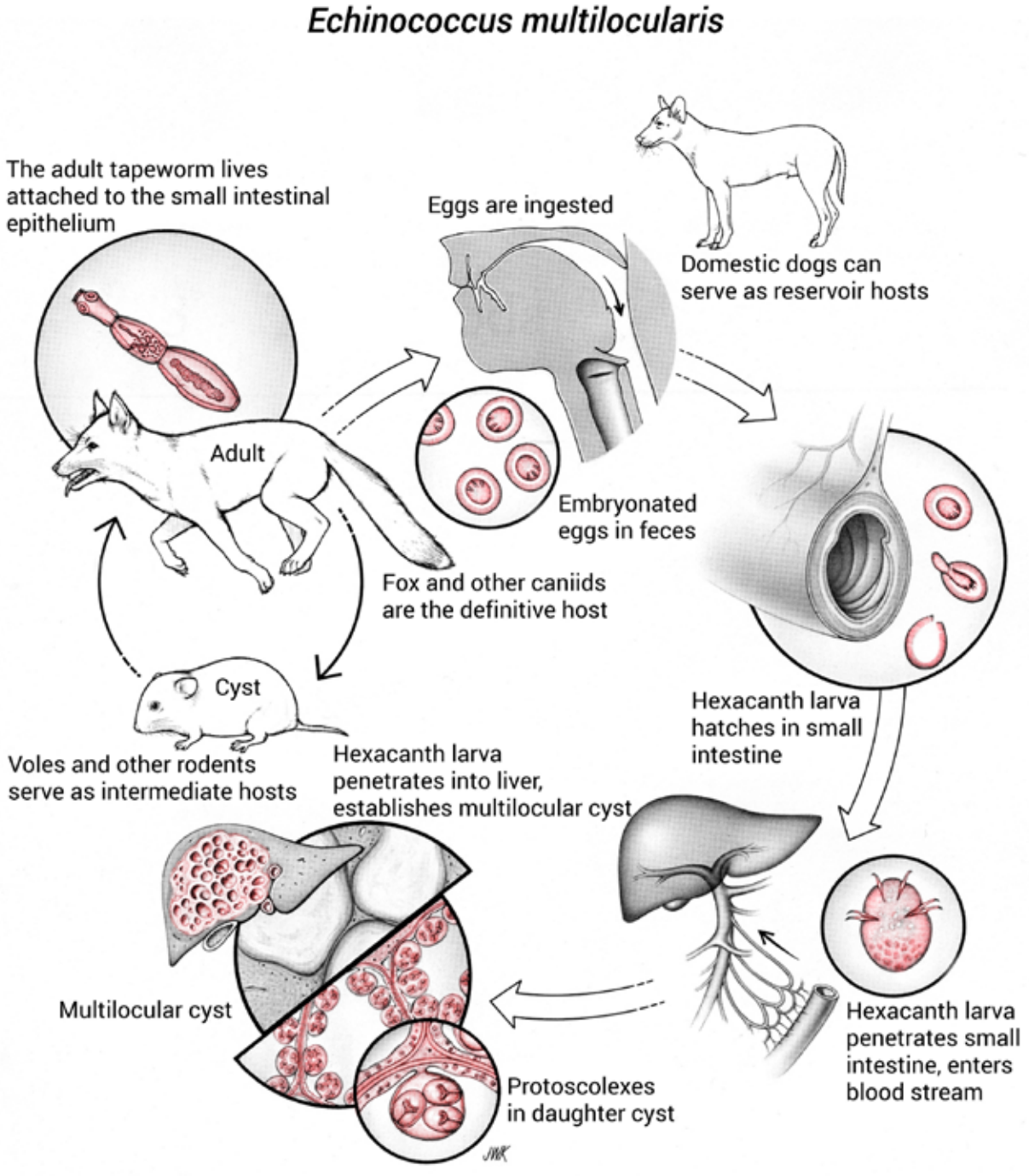
Strict regulations regarding the importation of animal products that might carry *E. granulosus* eggs (e.g., animal hides of various carnivores, fishing flies, etc.) is a requirement for this control strategy to be effective. A recombinant peptide vaccine, EG95, induces high levels of protection in sheep, and may prove useful in certain parts of the world.[60] Canine vaccination, which is currently being tested, may be critical in many areas of the world.[61, 62]

Echinococcus multilocularis

Pronunciation: \i-ˌkī-nə-ˈkä-kəs\\mul-tī-läk-yü-ler-is\

Echinococcus multilocularis (\eh-KYE-no-KOK-us\\MUL-tee-lok-you-lair-is\) infects wild Caenidae, such as fox, and can also infect domestic dogs. The intermediate hosts are usually rodents (e.g., voles, field mice, ground squirrels).[63] The biology of the infection in humans resembles the situation found in the intermediate 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.[64] This includes urban areas where reservoir hosts could 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 a number of Common Market countries.[65] It is also prevalent in the Russian Federation and 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.


Echinococcus multilocularis
The adult tapeworm lives attached to the small intestinal epithelium
Adult
Eggs are ingested
Domestic dogs can serve as reservoir hosts
Embryonated eggs in feces
Fox and other caniids are the definitive host
Cyst
Voles and other rodents serve as intermediate hosts
Hexacanth larva penetrates into liver, establishes multilocular cyst
Hexacanth larva hatches in small intestine
Hexacanth larva penetrates small intestine, enters blood stream
Multilocular cyst
Protoscolexes in daughter cyst
JWK

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, that grow as a single membrane-bounded unit. Foxes and dogs ingest infected liver containing numerous protoscolices, which leads to infection with the adult worm, thereby completing the life cycle.

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.[66] Alveolar disease can be highly aggressive, specifically 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 direct extension to the lungs and rarely metastasis to the CNS.[67] 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%.[64]

HLA type may play a role in a given individual's risk of developing further disease after chemotherapy. Re-growth of the parasite was significantly more prevalent in patients with haplotype HLA-DQB1*02 than those with haplotype HLA-DRB1*1157.[68] Interleukin-10 production was also higher in infected individuals, and may relate to the lack of development of protective immunity.[69] Other investigators have noted an important role of interleukin-5 and a robust Th2 response to infection with *E. multilocularis*.[70]

Diagnosis (see Clinical Appendix)

Diagnosis is based on radiographic imaging findings which show tumor-like lesions with areas of calcification.[71] Serologic testing by ELISA is both highly sensitive and specific and allows distinction between *E. multilocularis* and *E. granulosus*.[72, 73] Directed biopsy of the lesion can often establish the diagnosis.[63]

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.[64] Long-term adjuvant chemotherapy with albendazole or mebendazole improves the 10-year survival rate.[74, 75] Nitazoxanide has some demonstrated *in vitro* activity against *E. multilocularis* and may prove to be a useful alternative therapy.[76]

Prevention relates to the handling of 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. Use of oral baits laced with therapeutic doses of praziquantel for control of *E. multilocularis* infections in foxes has been effective in certain areas.[77] Monthly treatment of dogs at high risk with praziquantel may be an effective approach.

Mesocestoides spp.
(Valliant 1863)

Pronunciation: \me-zə-ses-ˈtȯi-dēz\

Introduction

Cestodes, in the genus *Mesocestoides* (\MEH-zo-SES-toy-DEEZ\), infect numerous species of mammals and birds. Human infections have been reported.[78] Most of the cases reported from the U.S. were caused by *Mesocestoides variabilis*.[79] 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 to an adult worm in the gut or migrate to the peritoneal cavity. Cases of *Mesocestoides* infection have been described in the U.S., but the mode of acquisition is not known.[78] A case of *Mesocestoides* 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.[79]

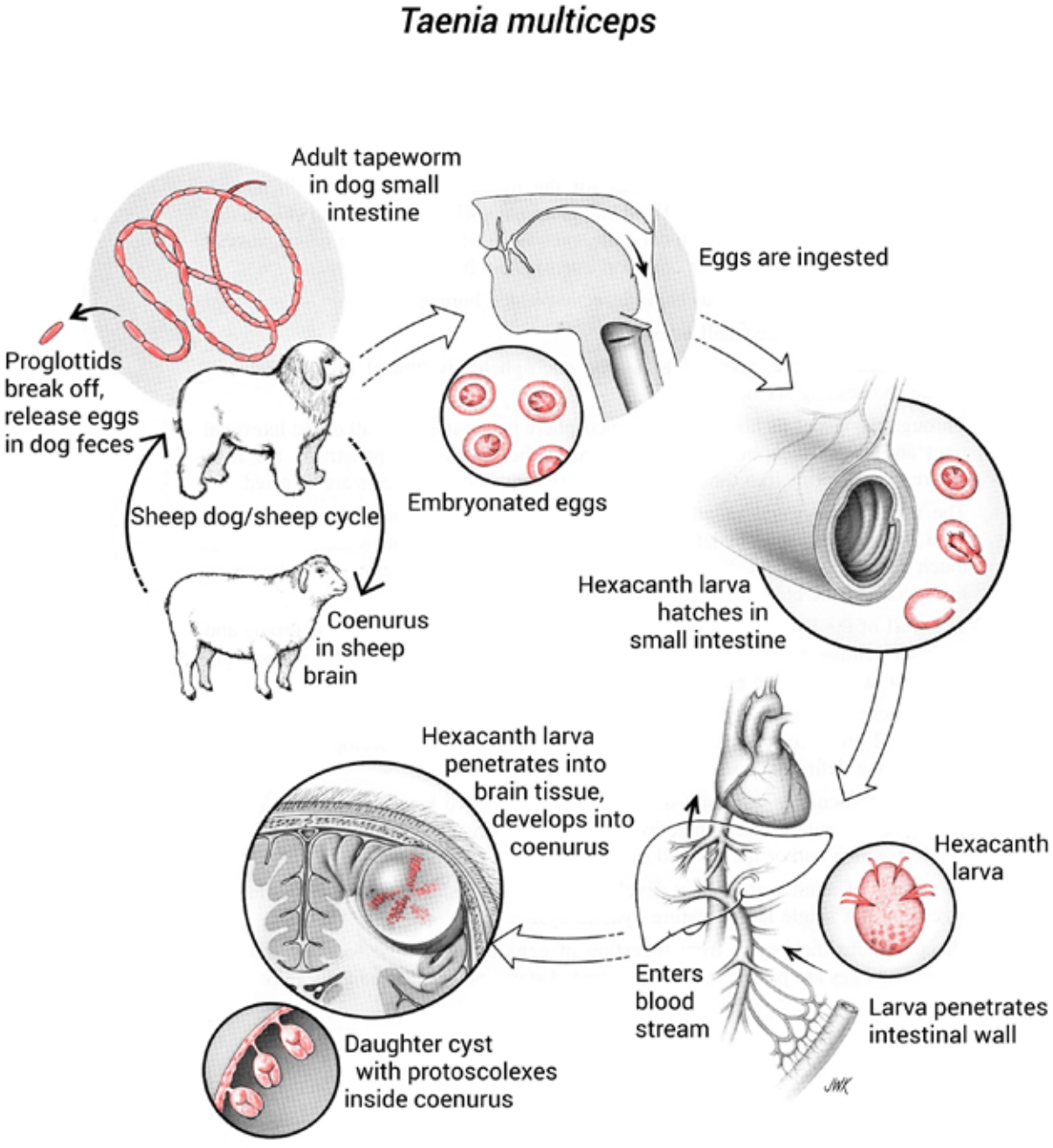
Spirometra spp.
(Mueller 1935)

Pronunciation: \spī-ˈräm-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.[80, 81] The definitive hosts for the many species of *Spirometra* are cats, birds, canines, and a number of other carnivores.[80, 82]

The life cycles of *Spirometra* spp. are complex and similar to that of *Diphyllobothrium 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.[83]

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*.[84, 85] The skin of numerous cold-blooded vertebrates contains a variety of closely related peptides, referred to as megainins.[86] These amphipathic peptides are related to gramicidin, and have potent anti-microbial activities.[87] Employing frog and fish skins for medical use has a chemotherapeutic basis. As with any other therapy, the use of cold-blooded vertebrate skin as a poultice can have unwanted "side effects." In this case, the user may develop a parasitic infection.


Taenia multiceps
Adult tapeworm in dog small intestine
Proglottids break off, release eggs in dog feces
Sheep dog/sheep cycle
Coenurus in sheep brain
Eggs are ingested
Embryonated eggs
Hexacanth larva hatches in small intestine
Hexacanth larva penetrates into brain tissue, develops into coenurus
Hexacanth larva
Enters blood stream
Larva penetrates intestinal wall
Daughter cyst with protoscolexes inside coenurus

The plerocercoid stage can migrate out of the poultice and into the subcutaneous tissues, stimulated by the rise in temperature from the human host. If the poultice is placed over an open wound, or the eye, the immature parasite may enter the site. In the eye, the inner surface of the lid is the initial site of infection.[88, 89] The surrounding tissues proliferate, becoming edematous and painful (unilateral peri-orbital edema), because the larva secretes a version of growth hormone similar to that of its mammalian host.[90] The pleurocercoid may migrate beyond the eyelid into the brain, where it continues to grow. Neurological symptoms follow.[91] Occasionally, patients die of neurological complications of infection with *Spirometra* spp..

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.[92, 93] In the case of early eye involvement, removal of the larva from the space between the lower lid and the eyeball results in 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.[94] In these instances, surgery is necessary. Subcutaneous lesions are often removed by surgery, as treatment with antihelmintic therapy such as with praziquantel is associated with limited success.[94] Prevention is difficult, because of the effectiveness and popularity of poultice use for a variety of medical problems, including photophobia due to chickenpox.[95]

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 cyclophyllidian 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 metacestode (juvenile stage), known as coenurus, which resembles cysts found in the intermediate hosts. Human infections have been largely confined to the African continent, but a few cases have been described from France, England, and North and South America.

The larva may invade the CNS (i.e., brain, eyes, spinal cord). Other space-occupying lesions resemble this infection, such as those caused by cysticerci and hydatid cysts.

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 antihelmintic therapies have been used, it is unclear how efficacious these are.[96] Recommendations for prevention are limited, because reservoir hosts include such a large number of species of wild animals.

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VII. The Trematodes

The class Trematoda, in the phylum Platyhelminthes, consists of two orders, Monogenea and Digenea. All are obligate parasites. Trematodes of medical importance only occur in the order Digenea, and include the blood flukes, the intestinal flukes, and the tissue flukes. They are mainly found throughout the tropics and subtropics, while a few species are encountered in temperate zones, as well.

Trematodes undergo complex developmental cycles in their intermediate and definitive hosts. But, as complex as trematode biology is, offering seemingly numerous opportunities for interrupting their life cycles, eradication of any medically important species has occurred to only a limited extent in specific geographic areas such as *Schistosoma mansoni* in China and *S. japonicum* in Japan.

Trematodes maintain their site location within the host using their 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 molecules. The tegument is covered by membrane-bound microvilli, underneath which are mitochondria, pinocytotic vesicles, and other structures facilitating nutrient acquisition.

In addition, trematodes have a functional blind gut into which they ingest tissues of the host. Ingested material is pumped down into the bifurcated intestinal tract, where digestion occurs, aided by enzymes (e.g., proteases, lipases, aminopeptidases, esterases). Since the gut has no exit, wastes are regurgitated into the host. These worms obtain a wide variety of nutrients in several different ways, making it difficult to develop drugs or immune-based therapies to interrupt metabolic processes.

Several layers of muscle lie just below the tegument, allowing trematodes to move about freely within the host. This activity can result in severe pathological consequences for the host, particularly in the case of *Fasciola hepatica, Paragonimus westermani* and the schistosomes.

A pair of dorsal ganglia gives rise to lateral peripheral nerves running the length of the body, and they innervate the muscle layers. Commissures from the lateral nerves also innervate various organs, including the gut, and reproductive organs. The flukes have no body cavity; rather, organs are embedded in the parenchyma.

In addition to solid wastes, trematodes excrete small molecular-weight compounds using a network of tubules that connect to collecting organelles. These, in turn, connect to the excretory pore at the tegumental surface of the parasite.

The trematodes in the order Digenea employ 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 complex, involving 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 secretions of the Mehlis' gland. It exits 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 are either stimulated to hatch in the external environment or, they hatch after being ingested by the next host. There, they undergo asexual reproduction, eventually increasing in numbers many-fold. Intermediate hosts include snail species that live in freshwater or terrestrial habitats. In addition, other invertebrates (e.g., insects, crabs) and a variety of cold-blooded vertebrates (e.g., fish) function as intermediate hosts for medically important species of trematodes. Plants (e.g., watercress, water chestnuts) are sites on which some species of metacercariae encyst.

Many species of adult trematodes are acquired by ingesting the intermediate stage (i.e., the metacercaria), but a few (notably the schistosomes) can actively penetrate unbroken skin. Site selection by trematodes within the human host is poorly understood. It is determined by a complex interplay between chemical and physical niches, which represent environmental cues from the host, and the receiving and translation of those cues by the nervous system of the parasite. Some drugs interfere with trematode nervous system functions (e.g., praziquantel), resulting in profound changes in worm behavior. Under those conditions, elimination of the parasite is possible.

33. The Schistosomes

Pronunciation: \shis-tə-ˈsō-mə

Schistosoma mansoni
(Sambon 1907)

Pronunciation: \man-sə-ˌnī\

Schistosoma japonicum
(Katsurada 1904)

Pronunciation: \jə-pän-c-əm\

Schistosoma haematobium
(Bilharz 1852)

Pronunciation: \hē-mə-ˈtō-bē-əm\

Schistosoma mekongi
(Bilharz 1852)

Pronunciation: \mə-käŋ-ē\

Schistosoma intercalatum
(Fischer 1934)

Pronunciation: \in-tər-kə-la-təm\

Introduction

Five 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-tum\), cause a series of related diseases in humans referred to as schistosomiasis. *S. intercalatum*, a parasite of cattle in West Africa, is a less common cause of disease in humans than the other schistosomes and is only associated with sporadic reports.

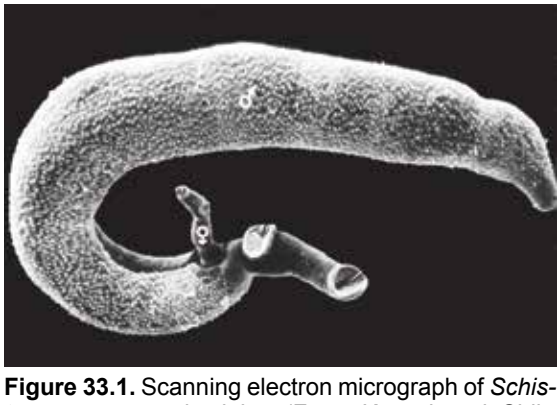
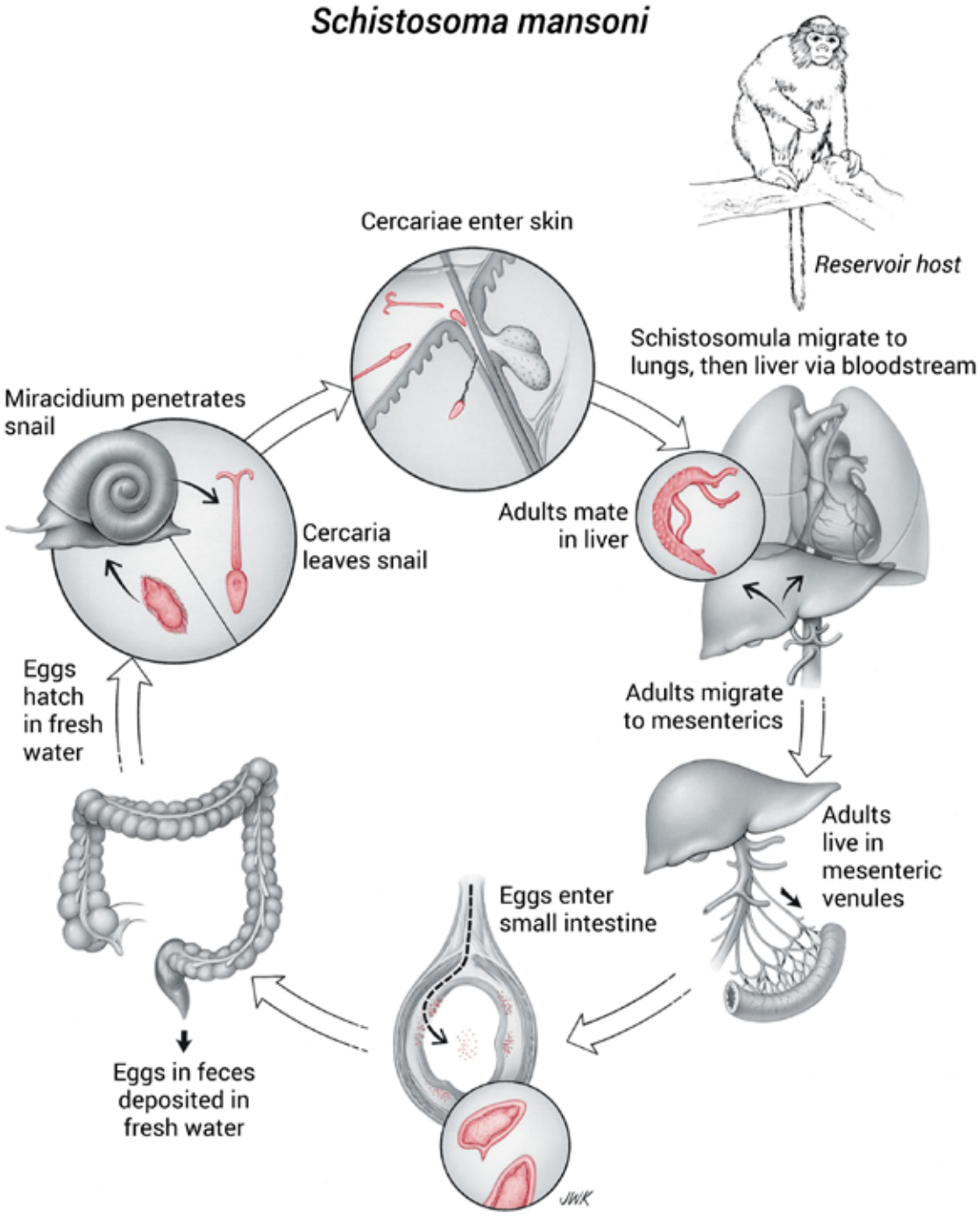


Figure 33.1. Scanning electron micrograph of *Schistosoma mansoni* adults. (From Kessel and Shih: Scanning Electron Microscopy in Biology. Springer-Verlag, 1976. Reproduced with permission).

Except for *S. haematobium* that produces urinary tract disease and female genital schistosomiasis (FGS), the human schistosomes primarily affect the intestine and liver. Chronic schistosomiasis also causes physical growth and cognitive delays in children.[1] The Global Burden of Disease Study in 2013 estimated that almost 300 million people suffer with some form of schistosomiasis, with approximately 90% of the cases found in Africa.[2] Additional estimates indicate that schistosomiasis causes up to 200,000 deaths each year.[3] In addition to those infected, a total of almost 800 million people are at risk worldwide.[4, 5] Schistosomes that infect humans are largely tropical in distribution, reflecting the geographical distribution of their intermediate host snail species. Poverty is a second critical factor, as it is for many human parasitic diseases.[6] 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 regional increases in schistosomiasis.

S. mansoni is found throughout most of Sub-Saharan Africa, Egypt and the Sudan, parts of the Middle East, some parts of South America (including Brazil, Venezuela and the Guyanas), and some islands in the Caribbean. It is the only form of schistosomiasis found in the New World and is believed to have been introduced over hundreds of years through the middle passage of the Atlantic slave trade.[7] Its intermediate hosts are aquatic snails in the genus *Biomphalaria*. Reservoir hosts for *S. mansoni* include baboons and monkeys in Africa. They play no significant role in the epidemiology of human disease.


Schistosoma mansoni
Reservoir host
Cercariae enter skin
Schistosomula migrate to lungs, then liver via bloodstream
Miracidium penetrates snail
Cercaria leaves snail
Adults mate in liver
Eggs hatch in fresh water
Adults migrate to mesenterics
Adults live in mesenteric venules
Eggs enter small intestine
Eggs in feces deposited in fresh water
JWK

S. haematobium is prevalent in most parts of Africa, and in some parts of the Middle East. An outbreak of *S. haematobium* was also reported in Corsica, France that was most likely initiated by an infected individual from Africa who had migrated there.[8, 9] Its aquatic intermediate host snails are in the genus *Bulinus*. There are no important reservoir hosts for this trematode species, although during an isolated outbreak in the Omo River Valley of Ethiopia among white-water rafters, the origin of the outbreak was traced back to monkeys.[10] *S. haematobium* causes urinary tract and FGS (urogenital schistosomiasis). In East and South African countries such as Tanzania, Zimbabwe, Malawi, Mozambique, and KwaZulu-Natal South Africa, urogenital schistosomiasis is a major co-factor in the HIV/AIDS epidemic.[11]

S. intercalatum occasionally infects people in Cameroon, Gabon, and Democratic Republic of Congo.[12-14]

S. japonicum occurs in China, the Philippines, and, to a small extent, Indonesia. It was eradicated from Japan in 1977.[15] Its amphibious intermediate host snails are in the genus *Oncomelania*. In contrast to the other schistosomes, zoonotic transmission occurs on a regular basis. There are important reservoir hosts for *S. japonicum*, including water buffalo, cattle and pigs.[16] *S. mekongi,* a closely related species, is found in the Mekong River in Southeast Asia. Although similar in morphology and life cycle to *S. japonicum, S. mekongi* is genetically distinct.[17]

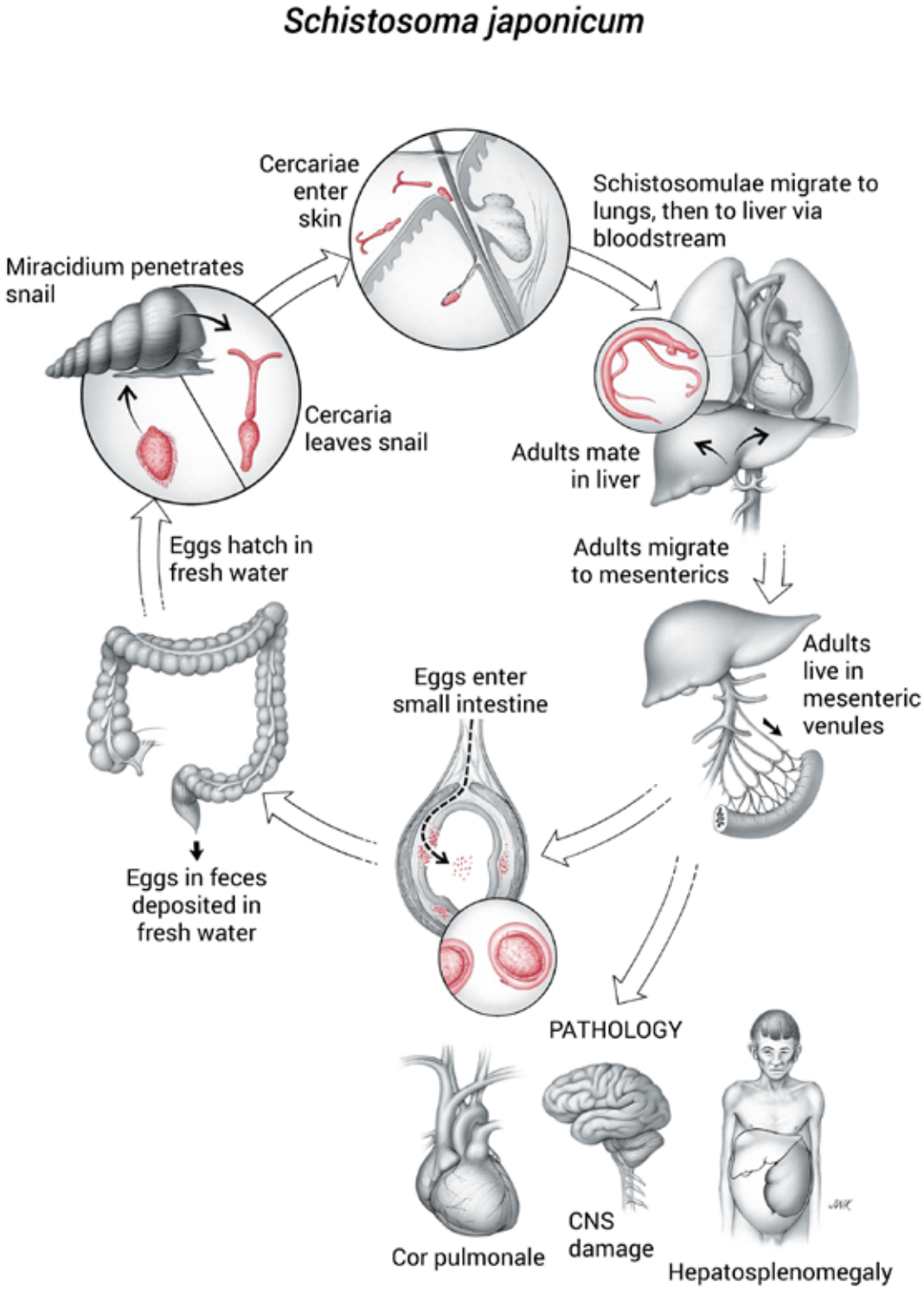
There are no autochthonous infections in the United States with any of the above species of schistosomes because there are no appropriate species of intermediate host snails, and, most importantly, sanitary disposal of feces and urine is the general rule. Thousands of Caribbean, African and Southeast 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

Paleoparasitologists have found *S. haematobium* eggs in mummies dating as far back as 1250 BCE.[18] It has been suggested 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, but there remains controversy about this despite possible references to *Schistosoma*-induced hematuria in early Egyptian papyri.[19] Hematuria in males, in fact, represents a late manifestation of *S. haematobium* infection.

In 1798, A.J. Renoult, a French army surgeon put forth the first modern description of what

Figure 33.2. Scanning electron micrograph of adult schistosomes. Notice gynecophoral canal with female inside. Photo D. Scharf.


Schistosoma japonicum
Cercariae enter skin
Schistosomulae migrate to lungs, then to liver via bloodstream
Miracidium penetrates snail
Cercaria leaves snail
Adults mate in liver
Eggs hatch in fresh water
Adults migrate to mesenterics
Adults live in mesenteric venules
Eggs enter small intestine
Eggs in feces deposited in fresh water
PATHOLOGY
Cor pulmonale
CNS damage
Hepatosplenomegaly

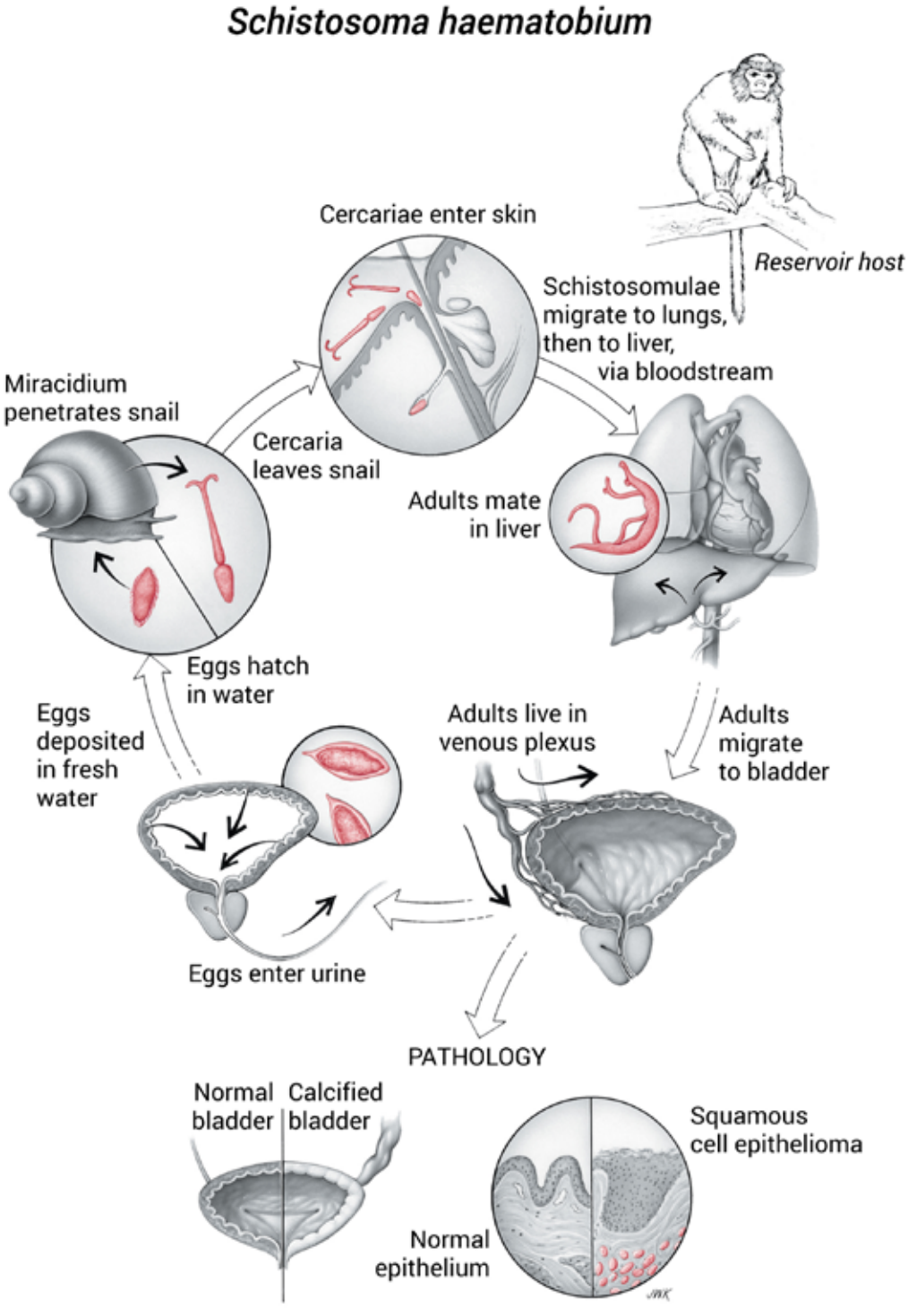
is believed to have been hematuria due to *S. haematobium*. Renoult described the epidemic of hematuria seen in Napoleon's soldiers who invaded Egypt.[20] Cases were subsequently described among troops involved in the Second Anglo-Boer War (1899–1902).[21] In 1851 and 1852, Theodore Bilharz and Ernst von Siebold reported human cases of *S. haematobium*, described the adult worm and made the connection with the appearance of hematuria.[22-24] They identified this as a parasite that occupied the venous plexus of the bladder and whose eggs possessed a terminal spine. In 1854, Wilhelm Griesinger described in detail the clinical disease and its pathology.[25] Griesinger 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.[26] He also carried out experimental infections with *S. haematobium* in various indigenous animals of northern Egypt, and proved that rats and mice were susceptible.

Although the symptoms associated with *S. haematobium* are perhaps easier to recognize 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, and in whose stool, but not his urine, he found many eggs with lateral spines.[27] In 1907, Louis Sambon recognized two blood flukes, on the basis of morphology and origin of the eggs in stool and urine.[28] In tribute to Manson, Sambon named this new organism after him, *Schistosoma mansoni*.[24] In 1908, Piraja de Silva also discovered *S. mansoni* in South America.[29] By 1918, Leiper had conducted extensive investigations on schistosomiasis, and reported the life cycle of *S. mansoni*, in which he described its snail intermediate host, and morphology of the adult worms.[26]

Giving credit for the discovery of *S. japonicum* is less straightforward. In 1888, Tokuho Majima described a case of cirrhosis and linked the presence of *S. japonicum* with this disease.[30] In 1904, Fujiro Katsurada described *S. japonicum* adult worms from infected cats.[31] At the same time, John Catto, working in Singapore, described an identical adult worm in a patient who died of cholera.[32] 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.[33] Kan Fujinami and Hatchitaro Nakamura in 1909, and Yoenji Miyagawa in 1912, independently reported on the details of the life cycle.[34, 35] In 1914, Keinosuke Miyairi and Masatsugu Suzuki, identified *Oncomelania* spp. snails as the intermediate hosts.[36]

S. japonicum infection has had a major impact on the history of modern China. It is believed that Mao's troops were unable to launch an amphibious assault on Taiwan in the late 1940s because they developed Katayama fever while encamped along the Yangtze River.[37] 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 by one.[38]

In the 1900s, a number of additional species of schistosomes, including *S. mekongi*, and *S. intercalatum,* were discovered and their life cycles, specific snail intermediates, and disease manifestations were described.[39]


Schistosoma haematobium
Reservoir host
Cercariae enter skin
Schistosomulae migrate to lungs, then to liver, via bloodstream
Miracidium penetrates snail
Cercaria leaves snail
Adults mate in liver
Eggs hatch in water
Eggs deposited in fresh water
Adults live in venous plexus
Adults migrate to bladder
Eggs enter urine
PATHOLOGY
Normal bladder
Calcified bladder
Squamous cell epithelioma
Normal epithelium

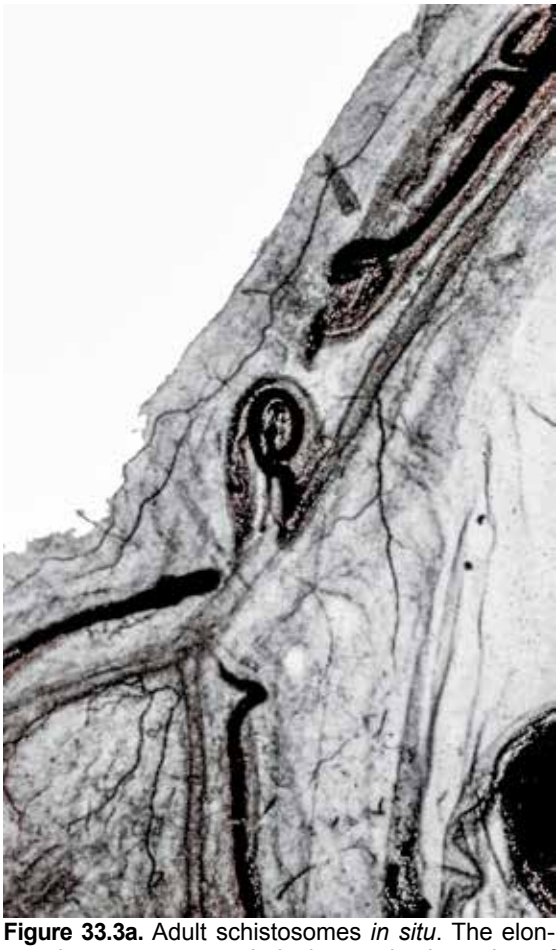


Figure 33.3a. Adult schistosomes *in situ*. The elongated worms appear dark due to the ingestion of hemoglobin.

Life Cycle

Schistosomes have separate sexes (Fig. 33.1); the female measures 15 mm in length, whilst the male is 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 the veins (Figs. 33.3a, 33.3b). *S. mansoni* lives in the inferior mesenteric veins that drain the intestine, 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 that drains the urinary bladder. The routes by which adult schistosomes arrive at these sites have been studied for various species using a number of different animal models, and is known to involve migration through various capillary beds.[40]

Worms live 5–8 years, on average, although some live as long as 37 years.[41] Schistosomes are facultative anaerobes, deriving energy primarily through the degradation of glucose and glycogen, and utilizing sophisticated transport mechanisms for glucose uptake and utilization.[42]

Adult schistosomes utilize hemoglobin as a primary source of amino acids, which is ingested into their blind, bifurcated gut.[25] They 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.[43] The female lies within the gynecophoral canal of the male (Fig. 33.2). This muscular, tegumental fold extends down both sides of the male, and may enable the female to feed on blood, by assisting in pumping blood into their esophagus. Single sex infections with

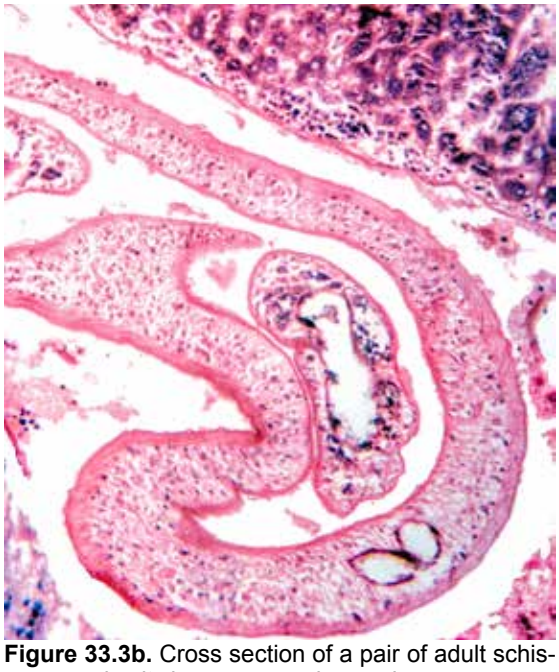


Figure 33.3b. Cross section of a pair of adult schistosomes *in situ* in a mesenteric venule.

Figure 33.4. Egg of *Schistosoma mansoni*. Note lateral spine. 150 μm x 60 μm.

females, or females experimentally separated from males, then re-introduced into the same host, do not produce eggs, presumably due to their inability to obtain a blood meal.

Free amino acids and glucose are transported across the tegument by active transport mechanisms. 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* have more widely spaced, shorter, finger-like projections, termed "tubercles". Evidence suggests that these projections have a sensory function.[44]

Females *in copula* lay eggs throughout their lives. The eggs of *S. mansoni* are oval, and possess a lateral spine (Fig. 33.4); those of *S. japonicum* and *S. mekongi* are globular and lack a large easily identifiable spine (Fig. 33.5a, 33.5b); those of *S. haematobium* are oval, with a terminal spine (Fig. 33.6). *S. mansoni* females produce, on average, 300 eggs per day, while *S. japonicum* and *S. mekongi* shed some 1500–3000 eggs daily. *S. haematobium* produce hundreds of eggs per day.[45] 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 penetrate into the surrounding connective tissue. The larvae, in the eggs, secrete lytic 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, eggs inadvertently escape into the circulation, which carries them to the liver via the portal circulation. Nearly 50% of all *S.*

Figure 33.5a. Egg of *Schistosoma japonicum*. No spine can be seen. 85 μm x 60 μm.

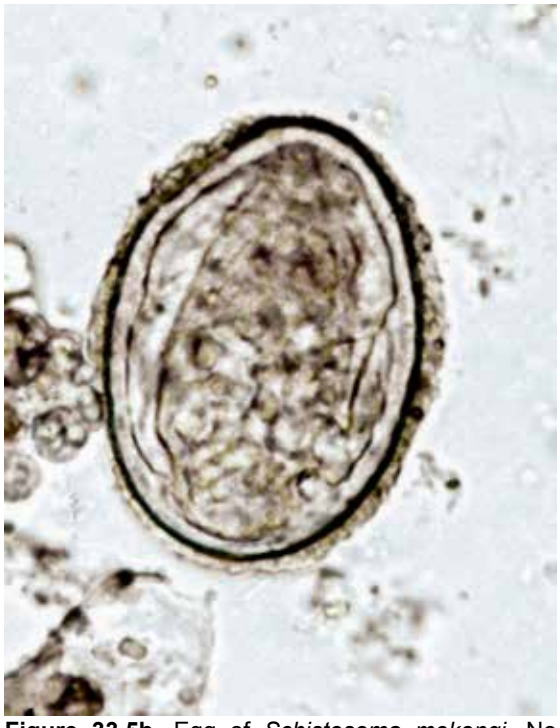


Figure 33.5b. Egg of *Schistosoma mekongi*. No spine can be seen. 65 μm x 50 μm.

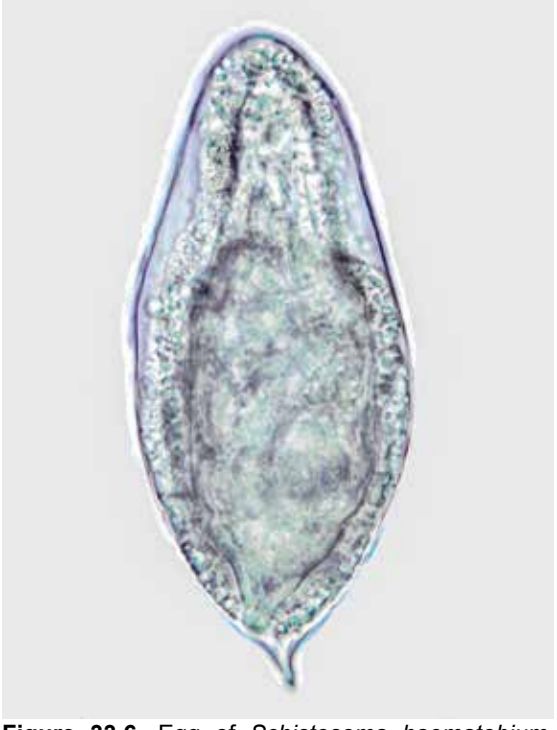


Figure 33.6. Egg of *Schistosoma haematobium*. Note terminal spine. 155 μm x 55 μm.

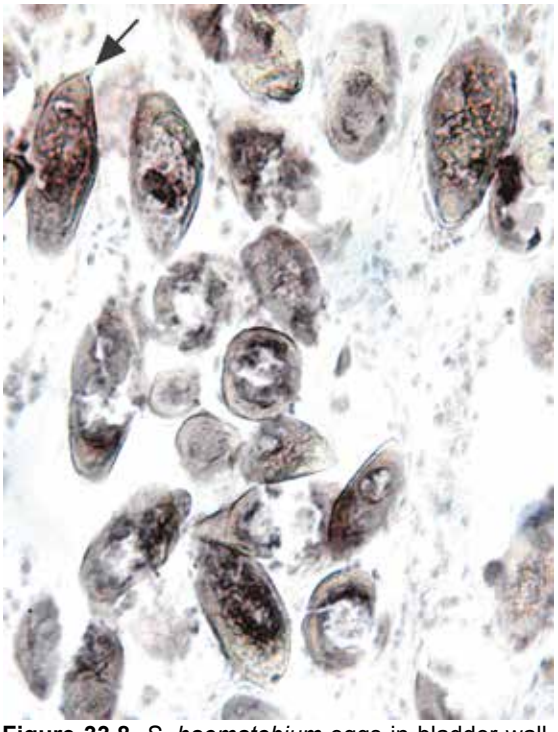


Figure 33.8. *S. haematobium* eggs in bladder wall. Note terminal spine (arrow).

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 both cases, the egg's penchant for penetrating tissues causes the infected individual significant pathological consequences.

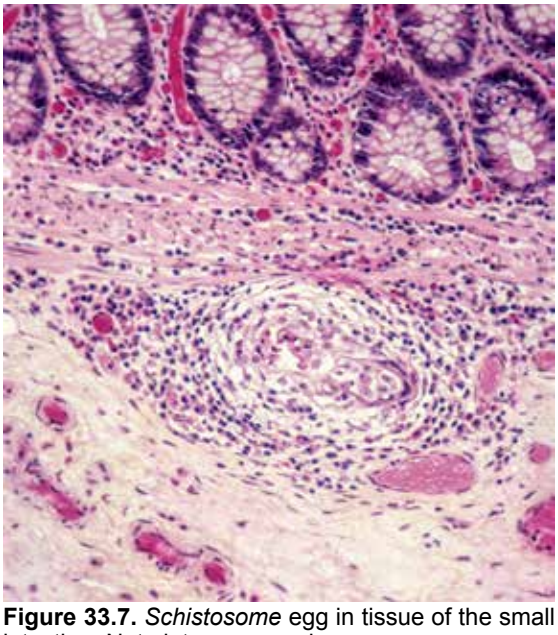


Figure 33.7. *Schistosome* egg in tissue of the small intestine. Note intense granuloma.

For the life cycle to continue, eggs in feces or urine must be deposited in freshwater. There, environmental cues trigger the larva stage, termed 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 proper snail host to do so. In essence, the snail is a chemical homing device for the parasite.

Upon finding the right 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 miracidium invades the snail and then the miracidium transforms into a primary (mother) sporocyst near the site of penetration. The primary sporocyst produces secondary sporocysts by asexual reproduction, and these migrate to the hepatopancreas.

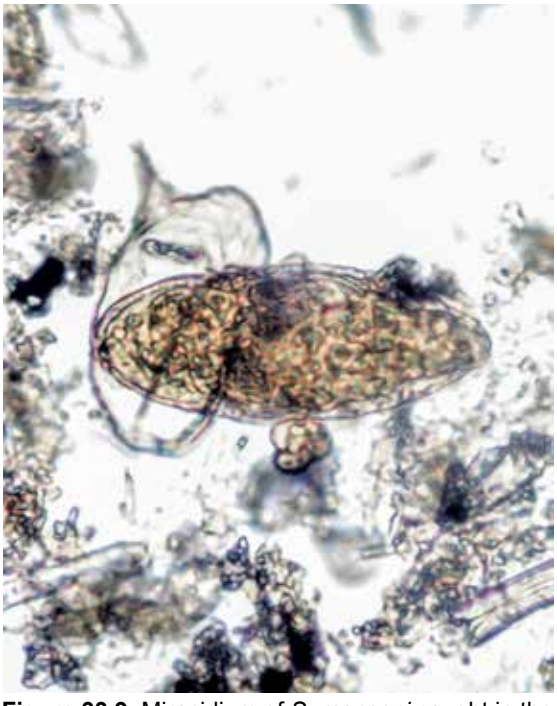


Figure 33.9. Miracidium of *S. mansoni* caught in the act of hatching.

Figure 33.11. *Biomphalaria glabrata*, the most common intermediate snail host for *S. mansoni*.

A series of remarkable transformations then ensue, beginning with production of the sporocyst. This stage gives rise to daughter sporocysts, which, in turn, produce cercariae, the infectious stage for humans. During each stage of development, there is an increase in the number of organisms. A single miracidium of *S. mansoni* produces some 4,000 genetically identical cercariae (Fig. 33.13). Throughout the process, the snail somehow manages to remain alive, even when it becomes infected with numerous miracidia. Each miracidium is either male or female, as are the resulting cercariae.

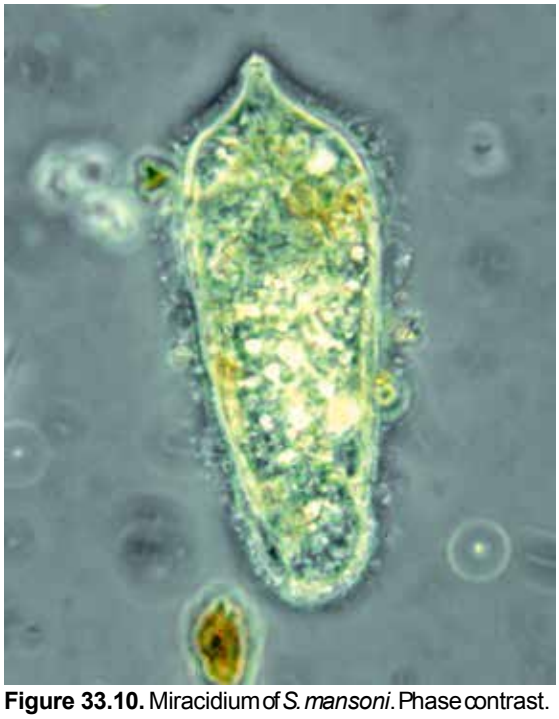


Figure 33.10. Miracidium of *S. mansoni*. Phase contrast.

Figure 33.12. *Oncomelania nosophora*, a snail intermediate host for *S. japonicum*.

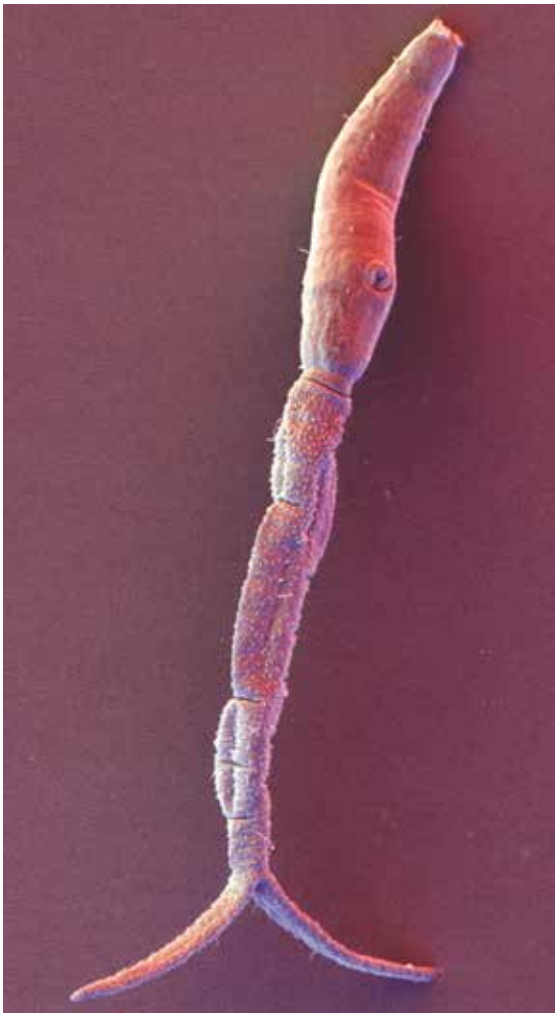


Figure 33.13. Scanning electron micrograph of a cercaria of *S. mansoni*. Photo D. Scharf.

Cercariae exit from the snail aided by yet another set of proteolytic enzymes. Cercariae are positively phototropic and negatively geotropic. They accumulate at the surface of water, and swim about seeking their definitive host by following gradients of chemical cues, including linoleic acid, that emanate from human skin. Cercariae must infect within 8 hours after emerging from its snail host; otherwise they exhaust their glycogen reserves and die.

Infection in the human host is initiated when the cercariae penetrate unbroken skin. With regards to *S. mansoni*, this step requires about half an hour, but occurs much more rapidly with *S. japonicum*.[46] Skin penetration is usually through a hair follicle, and is facilitated by release of another set of proteases and eicosanoids.[47] Cercariae shed their tails prior to penetration, and rapidly transform within the dermal layer of skin into the schistosomula stage. After approximately 2 days, the schistosomulae migrate through the bloodstream to the capillaries of the lung, where they remain for another several days. It is here that the immature worms acquire their ability to incorporate host serum proteins onto their tegumental surface. This "camouflage" has the profound effect of evading the immune system by appearing as "self". This enables the parasite to live out a long, and prosperous life inside its new host. 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 blood stream to the liver, where they mature to adult worms. Both sexes produce pheromones that are mutually attractive, and eventually worms of opposite sex find each other in the vastness of the parenchymal tissue. They mate there, and migrate out into the mesenteric circulation *in copula*. *S. mansoni* and *S. japonicum* worm pairs live in the small mesenteric veins that drain the intestines, while *S. haematobium* worm pairs live in the bladder venous plexus and possibly small veins that drain the female genital tract. Egg production begins shortly thereafter. 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

Adult schistosomes and the larval form usually do not cause significant pathological damage in the host because of their active

modulation of the host immune system.[48, 49] Evidence indicates that the adult worm pair elicits remarkably little in the way of host immunopathologic responses as a consequence of a unique cloaking strategy of antigen-masking their surface with host serum proteins that prevent the host from recognizing tegumental antigens.[50] They also shed tegumental components regularly, avoiding long-term exposure of that biologically active layer to its host immune surveillance system.[51] Adult schistosomes living in the venous circulation have the capacity to harbor enteric bacteria affixed to their surface. This relationship can result in the introduction of enteric bacteria, such as *Salmonella*, 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.[52]

In contrast to adults, the eggs produced by the worm pairs result in profound immunopathologic responses. This phenomenon accounts for almost all of the pathology and clinical manifestations of schistosomiasis. 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 schistosomiasis generally occurs only in people who suffer from recurrent heavy worm burdens. Increasing evidence suggests that a component of this phenomenon depends on host genetic factors.[53] In this regard, the same genes specific for susceptibility to *S. mansoni* have been identified in people living in Africa and South America.[54] In a study in the Sudan, a specific gene locus was associated with advanced liver disease confirming epidemiologic observations of fibrosis occurring in families.[55]

Furthermore, immunocompromised individuals with HIV shed fewer eggs in stool exams than similar individuals without HIV.[56] The soluble secretions from schistosome eggs, termed soluble egg antigens (SEAs), trigger host inflammatory and immune responses that result in granuloma formation, and are T cell-dependent, and include prominent Th2 components.[57, 58] Th2 bias downregulates other Th1 responses, and result in altered patterns of host susceptibility to other infectious pathogens, possibly including HIV.[59, 60] The pathogenesis of granuloma formation also requires host-derived production of TNF.[61] The diameters of the granulomas vary with the age of the infection. In newly acquired infections, granulomas are large, causing displacement of normal tissue with fibrotic, epithelioid reactions. Over time, eggs elicit

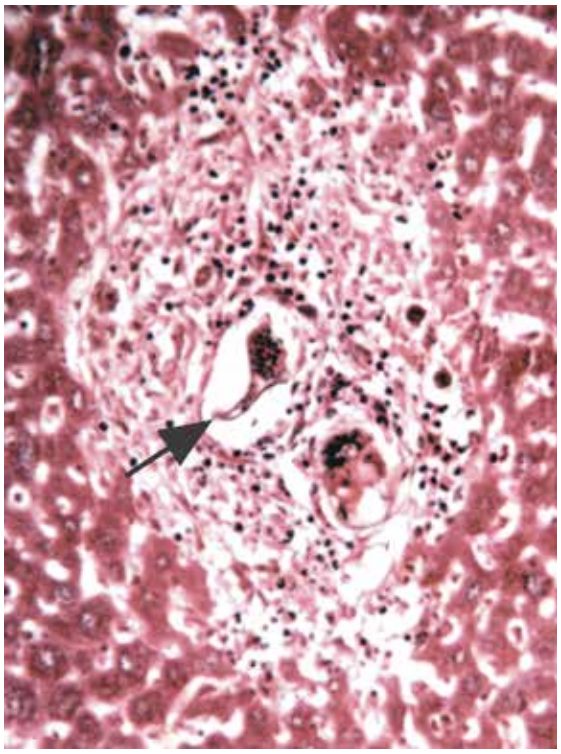


Figure 33.14. Granuloma in liver surrounding eggs of *S. mansoni*. Note the lateral spine (arrow).

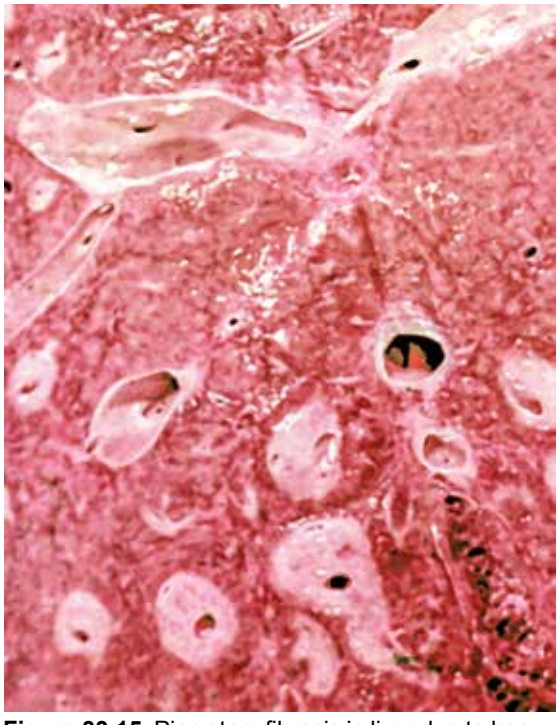


Figure 33.15. Pipe stem fibrosis in liver due to heavy infection with *S. mansoni*. Note normal liver tissue next to fibrotic vessels.

less and less volume of granulomatous tissue. This reaction appears to be under the regulation of interleukin-12.[62]

Granulomas form around eggs that collect in the intestinal wall and results in fibrosis. Erosion of the submucosa and villous 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.

Eggs swept back into the liver block presinusoidal capillaries, and induce granulomas there, as well. The presence of granulomas causes tissue fibrosis, and eventually leads to obstruction of the hepatic vasculature. Fibrosis of most of the portal areas incorporating the blood vessels leads to pipe stem fibrosis (Symmers' fibrosis) (Fig. 33.15), and, ultimately, to portal hypertension. Clinically, this manifests as hepatosplenomegaly, the extent

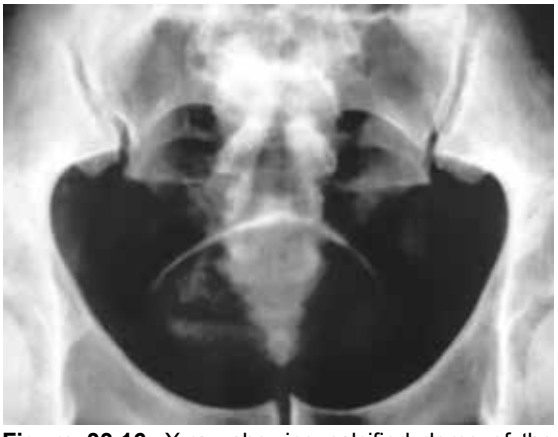


Figure 33.16. X-ray showing calcified dome of the bladder due to chronic infection with *S. haematobium.*

of which is dependent partially on host major histocompatibility class II alleles.[63] Development of collateral circulation follows, including esophageal varices. Parenchymal liver cells remain unaffected by granulomas and liver function remains normal.

Portal hypertension forces eggs to bypass the liver, and many are carried to the spleen, which becomes enlarged, further contributing to increased pressure in portal circulation. Infection with *S. japonicum* results in a greater number of granulomas, and consequently greater morbidity because this species produces, on average, five to ten times more eggs than *S. mansoni*. Collateral circu-

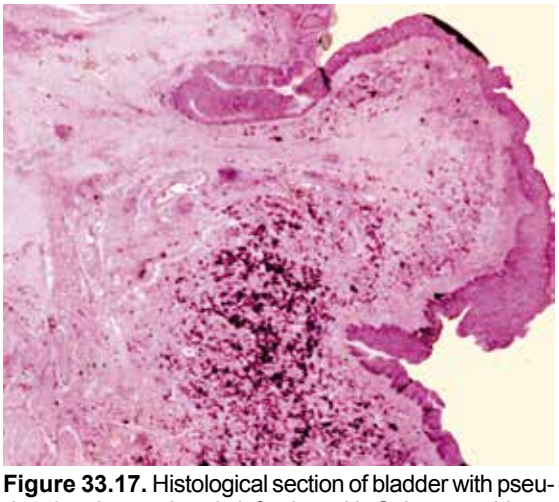


Figure 33.17. Histological section of bladder with pseudopolyp due to chronic infection with *S. haematobium.*

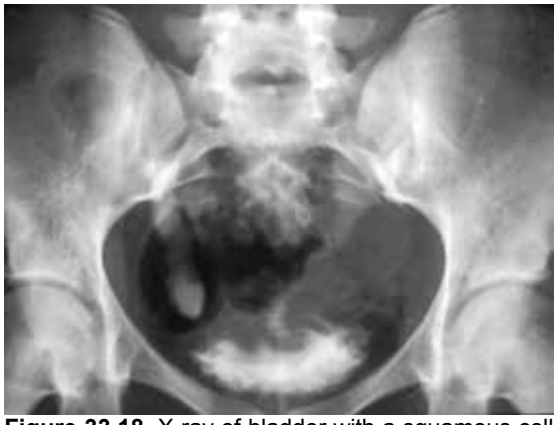


Figure 33.18. X-ray of bladder with a squamous cell tumor induced by *S. haematobium* eggs.

lation may also wash eggs into the lung capillary beds, occasionally leading to pulmonary fibrosis and *cor pulmonale*.

Accumulation of *S. haematobium* eggs around the bladder and ureters leads to granuloma formation and fibrosis. Calcification of dead eggs in the bladder wall (Fig. 33.16) results in rigidity of the bladder, and subsequent increased hydrostatic pressure in the ureters and kidneys. The bladder epithelium develops pseudopolyps (Fig. 33.17), which can transform into transitional squamous cell carcinoma in untreated patients (Fig. 33.18).

In some patients with long-standing disease (in all major types of schistosomiasis), deposition of immune complexes in kidneys can lead to basement membrane disease.[62]

Emerging evidence over the last decade confirms that *S. haematobium* eggs also gain access to the female genital tract to cause FGS. Granulomas in the uterus, cervix, and vagina produce ulcerative lesions, which are rich in inflammatory cells. These lesions presumably provide conduits for the entry of HIV during intercourse.[11] Through such mechanisms, *S. haematobium* infection is linked to a 3–4-fold increase in acquiring HIV/AIDS.

Penetration of the skin by cercariae is dependent on the release of parasite-derived proteases and eicosanoids. The process of host

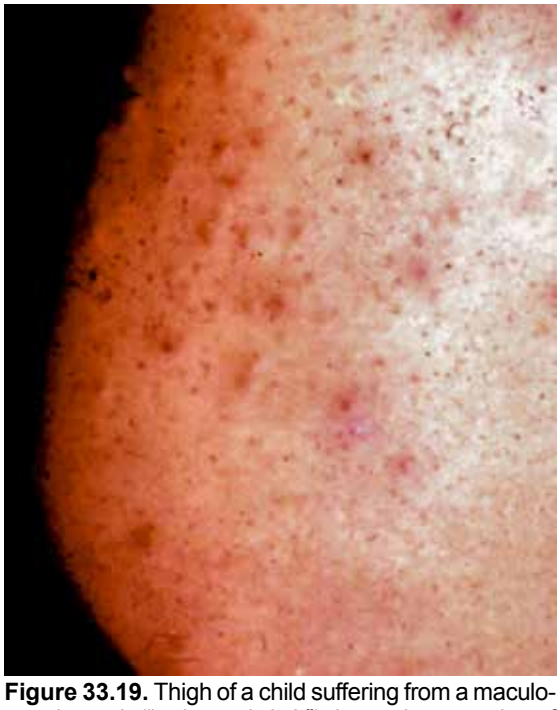


Figure 33.19. Thigh of a child suffering from a maculopapular rash ("swimmer's itch") due to the cercariae of a schistosome species that normally infects birds.

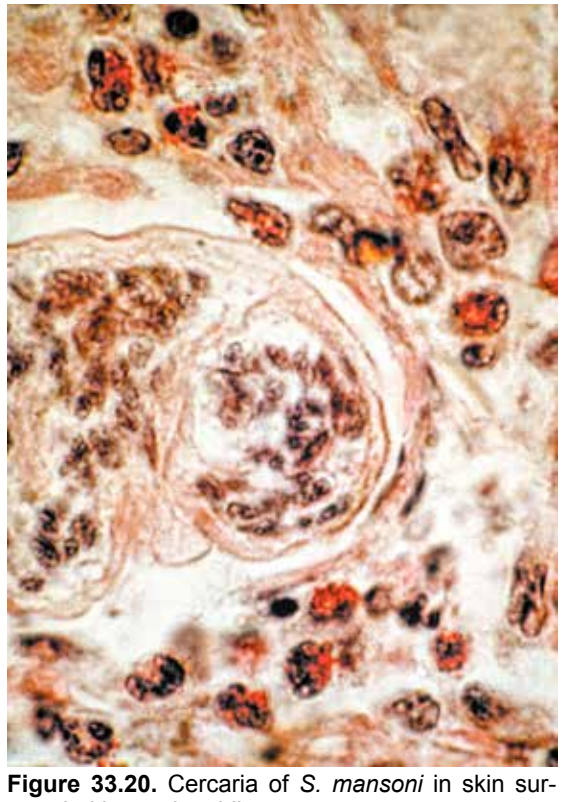


Figure 33.20. Cercaria of *S. mansoni* in skin surrounded by eosinophils.

entry typically causes no major reaction, but repeated exposure can lead to sensitization, and the development of a maculopapular rash (Fig. 33.19), characterized by IgE or IgG antibodies and an eosinophilic infiltrate. This is particularly true of accidental skin penetration by avian or bovine schistosomes.

Many schistosomes specifically parasitic for animals, can cause aberrant infections in humans. Avian schistosomes of the genera *Austrobilharzia*, *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 both penetrating cercariae and migrating schistosomulae are a critical component of naturally acquired immunity to human schistosomiasis. This derives from experimental evidence showing 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 being able to transform into schistosomulae. In so doing they elicit protective immune responses, including interleukin-13.[64-66] These observations are the basis for an experimental vaccine in non-human primates. The cercariae must remain alive in order to secrete the antigens associated with vaccine protection. In humans living in endemic regions, a protective process may take years of exposure to cercariae. Until then, young children have a particular problem mounting an effective immune response to invading schistosomulae. The mechanism by which children during their early years of exposure to cercariae and invading schistosomulae are susceptible to the parasite, but then become resistant over time is unclear, but appears to be due to age-related aspects of the innate immune response.[45, 67] One hypothesis is that young children respond initially to the parasite by producing IgG_4 blocking antibodies.[68] It has been suggested that blocking antibodies delay the development of protective IgE that is needed for the resistance to infection that older people have developed in endemic areas.

Current understanding of Th1 and Th2 immune mechanisms help inform the selection of recombinant schistosome protein antigens to use in the production of a practical vaccine.[69, 70] Animal protection studies have used several naturally purified or recombinant proteins such as paramyosin, calpain, tetraspanin, and others with good results in a mouse model, although the mechanism of protection is still under investigation.[71, 72] Studies in the Philippines in a population with risk of exposure to *S. japonicum* demonstrated that individuals with predominantly Th1 cellular immune responses appeared resistant to initial infection.[73, 74]

Clinical Disease

As in other helminth infections, clinical disease resulting from schistosomes usually occurs only in heavily infected individuals. The clinical manifestations of acute schistosomiasis occur predominantly in *S. japonicum* and *S. mansoni* infections. This condition is sometimes known as "Katayama fever". The classical disease attributed to schistosomiasis occurs during chronic infections. Chronic infection with *S. haematobium* can also 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 levels of exposure to either *S. japonicum* or *S. mansoni* cercariae. The name reflects the early descriptions of this syndrome in the

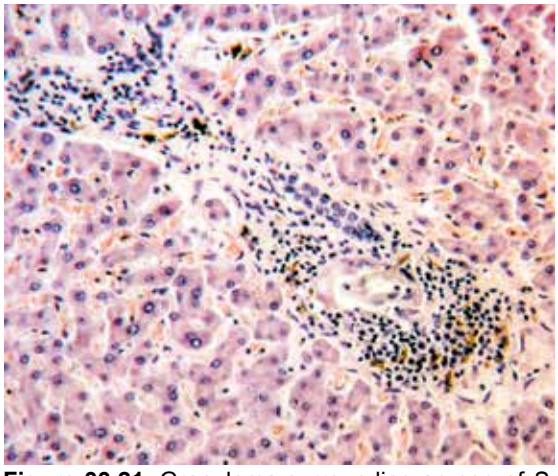


Figure 33.21. Granuloma surrounding an egg of *S. mansoni* in liver tissue.

Katayama District of Japan.[75] The symptoms are often dramatic and appear approximately 4–8 weeks after initial exposure, when adult worm pairs begin releasing their eggs in the tissues. Some investigators believe that Katayama fever resembles some of the manifestations of serum sickness. There is also a clinical resemblance to typhoid fever. Patients experience hepatosplenomegaly and lymphadenopathy as well as an 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 as a consequence of 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 in the liver parenchyma. With *S. haematobium*, injury occurs in the bladder.

The extent of injury depends on chronic worm burden, so chronic schistosomiasis occurs predominantly in individuals who are predisposed to repeated heavy infections.[68] In a population with repeated high exposures to cercariae, less than a quarter develop heavy infection and only 10% of these individuals with heavy infection 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).[76] It is partly reversible by specific anthelmintic therapy.[77]

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 development of a 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, long standing infections can cause nephrotic syndrome, resulting from the deposition of immune complexes onto the glomerular membrane.

S. haematobium, unlike the other three major schistosomes, 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 as well as 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 an 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 regions where *S. haematobium* is endemic. In contrast to adenocarcinoma, 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* are able to induce this through a number of mechanisms including the action of estrogen metabolites.[78] Squamous cell carcinoma is the most common type of bladder cancer in parts of Egypt, as well as elsewhere in Africa. Over time, it is possible that *S. haematobium* eggs may function as a human carcinogen that elicits metaplastic changes in the bladder.[79]

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 on sexual intercourse. On colposcopic exam FGS presents as "sandy patches" that correspond to the presence of schistosome granulomas. These patches bleed easily on contact. FGS also has important 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 major co-factor in Africa's AIDS epidemic.[11]

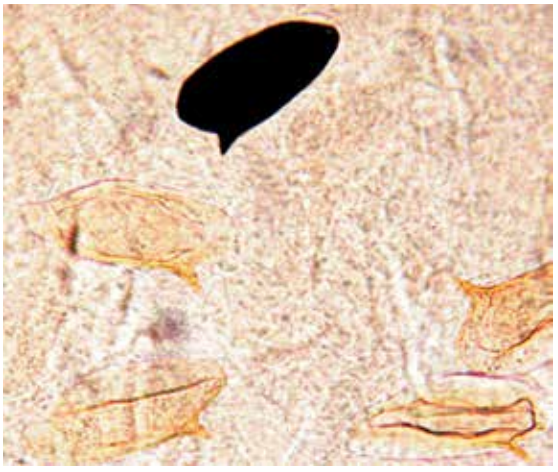


Figure 33.22. Biopsy of rectal tissue revealing eggs of *S. mansoni*. Note calcified egg, indicating that the infection was chronic.

CNS schistosomiasis

Rarely, schistosomes induce focal inflammatory reactions within the central nervous system (CNS), caused by deposition of eggs in the spinal cord and the brain.[80] *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 through detection of the parasite or the host 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, concentration of a specimen collected over a 24-hour period is required, because the number of eggs in stool can be few. Quantitative egg counts are sometimes useful for epidemiologic studies attempting to determine infection intensities.

For light infections, or in patients from whom egg excretion is intermittent, and from whom eggs cannot be found in stool, a rectal biopsy can be carried out (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 then be observed under higher power and examined for the presence of "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, which would make the identification of live miracidia in eggs impossible.

While most schistosome eggs appear in feces, urine should be examined for the presence of eggs of *S. haematobium* if this species is suspected. The urine sample should generally be collected close to noon, when egg excretion is usually maximal. 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.

Diagnosis of FGS requires training to identify the characteristic sandy patches associated with this condition. Confirmatory microscopy is a useful aid but not always available in resource-poor settings.

A number of additional tests have become available for the diagnosis of schistosomiasis. Two schistosome glycoprotein antigens known as CCA and CAA that circulate in the bloodstream of acutely-infected patients have been identified and can now be tested for using both qualitative and quantitative assays that are currently available.[81-83] These assays can be easily applied to urine samples.[84, 85] A number of NAATs have been developed that allow for detection as well as schistosome speciation.[86-88]

Antibodies develop 6–12 weeks after exposure and tend to become positive before eggs are evident in urine or stool.[89] There are a number of serological tests available with western blot confirmatory testing, as well.[90] Since serologies remain positive for long periods after treatment of infection, they cannot be used for follow-up and are difficult to use to monitor repeat infections in endemic areas.[91]

Portable ultrasound imaging has been shown to be clinically useful in the diagnosis of schistosomiasis. Ultrasound can define the extent of Symmers' fibrosis in patients with *S. mansoni* or *S. japonicum* infections, while the chronic obstructive changes associated with *S. haematobium* infection can also be detected.[92]

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 the acute schistosomiasis syndrome is a hypersensitivity reaction to the parasitic antigens, anthelmintic therapy results in exacerbation of symptoms in about half of those treated.[93] 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.[93, 94]

Acute treatment with anthelmintic therapy is not required to prevent the chronic manifestations of schistosomiasis.[95] How long to

wait after the acute syndrome subsides before initiating anthelmintic therapy is unknown, but it seems 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.[95] Treatment can then be repeated 4–6 weeks after this first treatment course.

For treatment of 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 greatly reduce the worm burden in individuals in endemic areas with high worm burdens, and to cure those with low worm burdens.[96-98] 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.[99] In younger patients, praziquantel may also reverse some of the pathology associated with Symmers' fibrosis.[100] There is evidence that part of its effectiveness is due to synergism with the host's humoral immune response.[101] Because it is effective in a single dose, it has been used in control programs (see below).

For patients no longer in endemic areas, test of cure is recommended with repeat testing for eggs in urine or stool no sooner than 3–6 months after treatment.[102] When treatment is initiated patients should be monitored for evidence of CNS disease, as treatment can cause an acute inflammatory response in patients who have eggs in the CNS. If this develops, prompt and prolonged therapy with corticosteroids is critical to prevent worsening of neurological symptoms and irreversible damage.[103, 104]

Praziquantel is now inexpensive and the World Health Assembly has endorsed community treatment of school-age children in endemic areas. The Schistosomiasis Control Initiative based at Imperial College, London, is leading global efforts to provide mass drug administration of praziquantel. Currently, praziquantel is being provided in "rapid impact" interventions, which include mass-drug treatments for intestinal helminth infections, lymphatic filariasis, and onchocerciasis.[105] Political obstacles have so far failed to link this approach with antiretroviral drug therapy for HIV/AIDS. Millions of girls and women living in poverty in Africa have been denied access to an inexpensive approach to HIV/AIDS prevention.[106] Resistance has occurred and a number of treatment failures have been reported.[107-109]

Alternatives to praziquantel are limited in use due to a higher frequency of adverse reactions and differences in spectrum of activity. Oxamniquine is an alternate drug with good anti-parasitic activity. In some regions, oxamniquine is as effective as praziquantel for the treatment of infections with *S. mansoni*, and metrifonate is effective for the treatment of *S. haematobium* infections.[110] The anti-malarial drug artemether has been studied in China as a chemoprophylactic agent in patients who anticipate high levels of exposure to *S. japonicum* and *S. mansoni* cercariae during seasonal floods. Chemoprophylactic activity of artemether was present but lower against *S. haematobium.*[111] 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.[112]

Treatment should be carried out only in patients with active schistosome infections. Portocaval or splenorenal shunts should be avoided in untreated schistosomiasis, because

Figure 33.23. Lake Nasser and the Aswan High Dam in Egypt. Photo S. Musgrave, astronaut *extraordinaire*.

they increase the probability of eggs reaching the lungs. If such a shunt is mandated by the intensity of portal hypertension, it should be carried out only after treating with any of the above-mentioned drugs.

Prevention and Control

Schistosomes' success in carrying out their life cycles is dependent upon complex ecological interactions with a wide variety of invertebrate and vertebrate host species. They appear to have numerous 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.[113-116] Although more challenging for less-developed countries, a number of programs including mass treatment to reduce worm burden have improved the situation for many.[117, 118]

Prevention of schistosomiasis by individuals requires that they never come in contact with infested freshwater. This suggestion is impossible to carry out in much of the world because of many complex economic, cultural, and behavioral patterns. In addition, it may be necessary for many people to be in contact with freshwater 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).

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.[119, 120]

Control of *S. japonicum* is complicated by the occurrence of reservoir hosts, such as water buffalo and cattle, in many regions of Asia. In Japan, this problem was overcome mainly by eliminating the use of water buffalo in rice farming and using horses (a non-susceptible host) as draft animals instead.[15]

The gold standard of control for schistosomiasis has been mass-drug administration of praziquantel, with the London-based Schistosomiasis Control Initiative leading the way to providing 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, DFID (United Kingdom), and some private donations, including an innovative New York-based END Fund, and a Washington DC based END7 campaign based at the Sabin Vaccine Institute.

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 frequently not possible in poor, developing rural areas without the support of the international community.[121] Control of the infection with anthelmintic drugs alone is difficult. There is also concern about the emergence of praziquantel drug resistance.[122]

Investigations into vaccinations and new therapeutics are currently underway and offer hope for improved methods to help address this neglected tropical disease.[45,62,69,71,72,123-129]

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34. *Clonorchis sinensis* and *Opisthorchis* spp.

Clonorchis sinensis
(Looss 1907)

Pronunciation: \klō-ˈnȯr-kəs\ \sin-en-səs\

Opisthorchis viverrini
(Poirier 1886)

Pronunciation: \äp-əs-ˈthȯr-kəs\ \vi-ver-rē-nē\

Opisthorchis felineus
(Rivolta 1884/Blanchard 1895)

Pronunciation: \äp-əs-ˈthȯr-kəs\ \fel-in-ē-əs\

Introduction

There are three major fish-borne liver flukes of major significance for human health: *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 mostly 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 is also a major liver fluke species. *O. felineus* can be found in Siberia, and a focus has also been identified in Italy.[1, 3-6] The biology, pathogenesis, and clinical disease of all three species are similar, so in most of this chapter *C. sinensis* will be presented as the model organism for fish-borne liver flukes affecting humans. On occasion the unique differences of *O. viverrini*, and *O. felineus* will be mentioned.

C. *sinensis* has numerous reservoir hosts, including dogs and cats. More than 25 million people in the Far East are infected with these fish-borne liver flukes, and some estimate that up to one fourth of Chinese immigrants to the United States harbor these flukes.[2, 7] Approximately 10 million people in northern Thailand are infected with *O. viverrini*, 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 major 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 in Calcutta, India, and Arthur Looss renamed

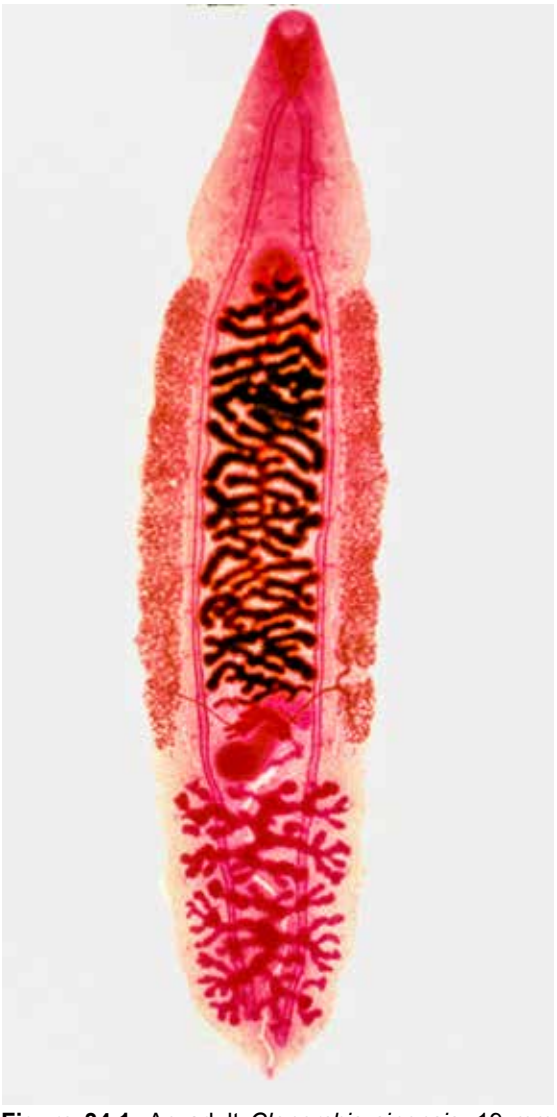
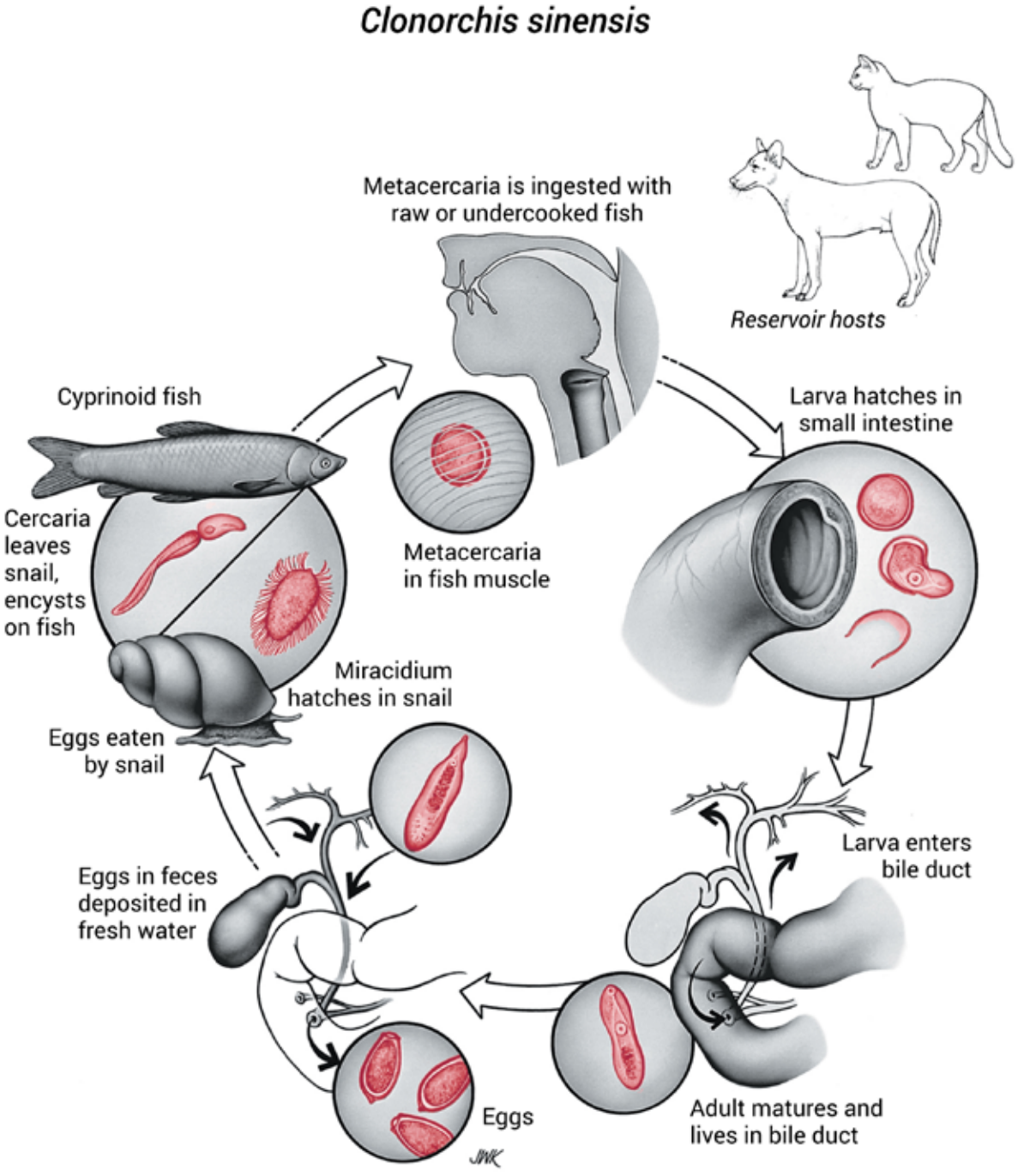
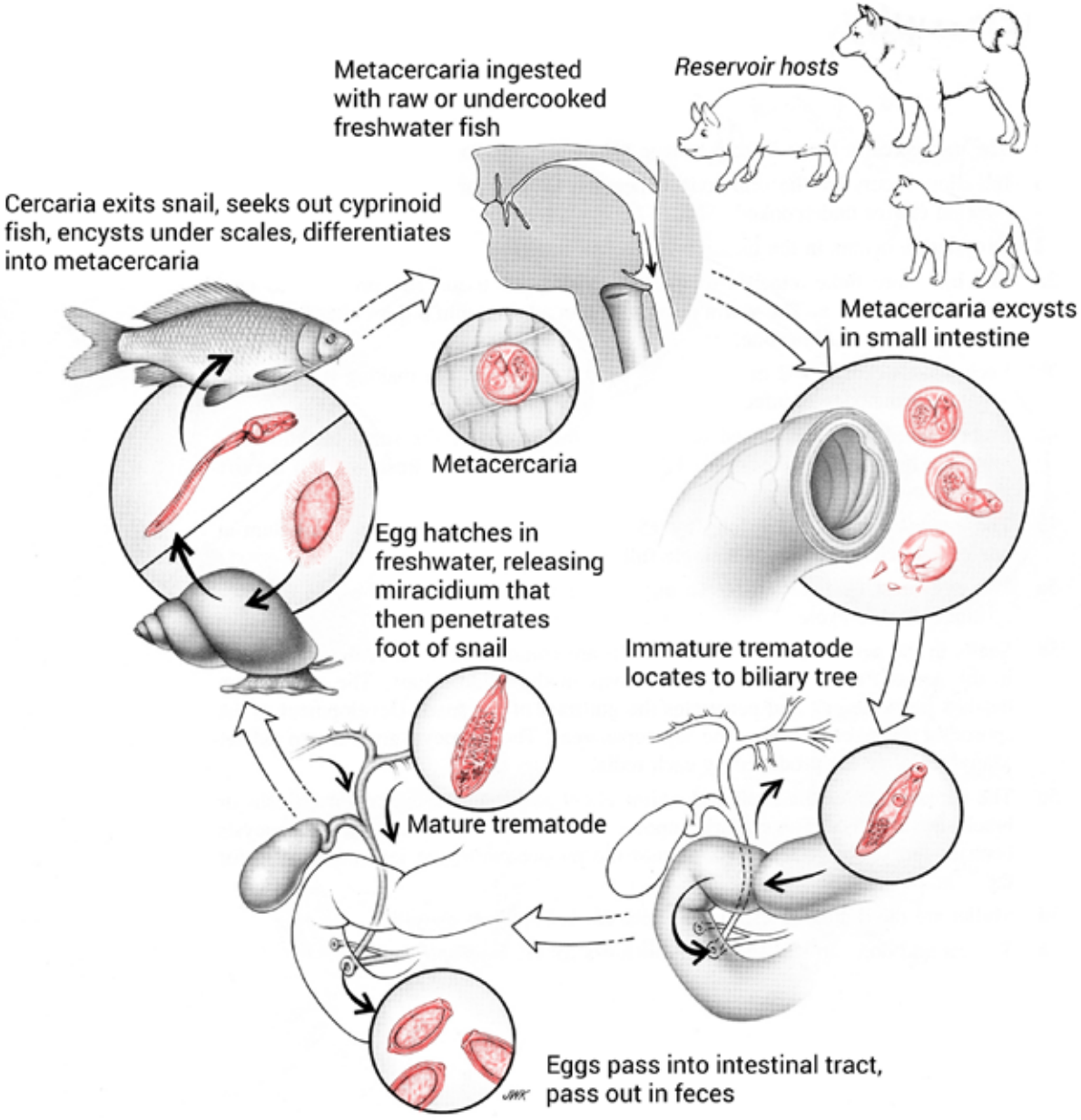


Figure 34.1. An adult *Clonorchis sinensis*. 19 mm x 3.5 mm.


Clonorchis sinensis
Metacercaria is ingested with raw or undercooked fish
Reservoir hosts
Cyprinoid fish
Larva hatches in small intestine
Cercaria leaves snail, encysts on fish
Metacercaria in fish muscle
Miracidium hatches in snail
Eggs eaten by snail
Larva enters bile duct
Eggs in feces deposited in fresh water
Eggs
Adult matures and lives in bile duct

Opisthorchis viverini



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 snail 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]

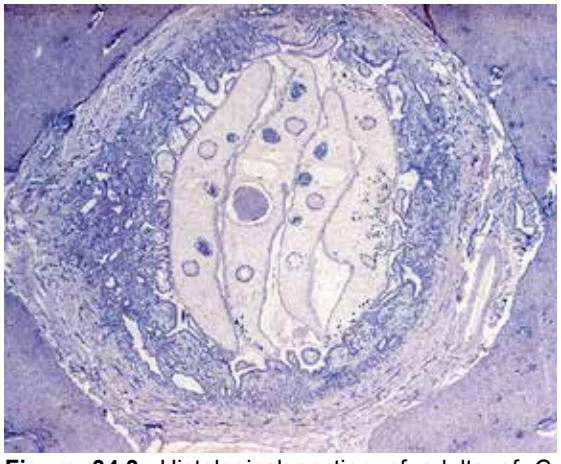


Figure 34.3. Histological section of adults of *C. sinensis* in bile duct.

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] There are multiple species of freshwater fish that may harbor this parasite and, despite referring to these as fish-borne liver flukes, 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 the bile duct (Fig. 34.3), remaining there, 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 epithelium. Each parasite may live for up to 26 years in the biliary system.[20] Since each worm has both male and female reproductive organs, self-fertilization is the norm. Single worms are capable of producing eggs without the requirement of finding a mate. Egg production follows self-fertilization. Embryonated eggs (Fig. 34.4) pass

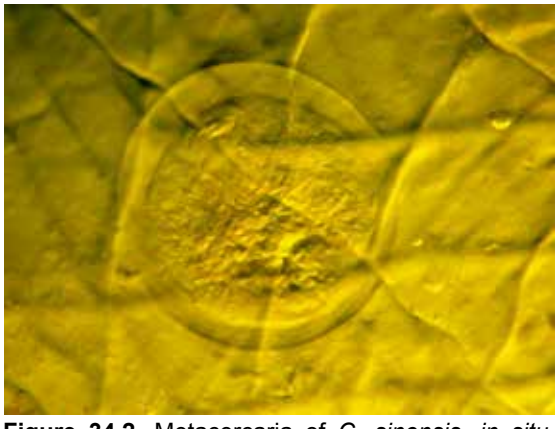


Figure 34.2. Metacercaria of *C. sinensis*, *in situ*, under the scales of a grass carp. 165 μm.

Figure 34.4. Eggs of *C. sinensis*. 30 μm x 15 μm.

from the common bile duct into the small intestine and are excreted with the feces. These eggs must reach freshwater in order 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 to 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, as well the introduction of pyogenic bacteria into the infection sites.[27] Through this process, chronic liver fluke infection can result in recurrent ascending cholangitis and pancreatitis.[16, 28-31] Over time, the presence of these fish-borne trematodes in the biliary tree may result in squamous metaplastic changes that lead to cholangiocarcinoma.[32] This is particularly true of heavy *O. viverrini* infections in Thailand that can be associated with a 15-fold increase in 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 unravelled.[33]

Clinical Disease

The symptoms seen in patients both acutely and chronically infected by these fish-borne trematodes are mostly determined by inoculum of metacercariae and worm burden. In acute infections with few metacercariae, patients are often asymptomatic, while patients infected with large numbers of metacercariae may present with right upper quadrant abdominal discomfort and tenderness, nausea, diarrhea, and headache.[34]

Heavy chronic infections can result in hepatomegaly, right upper quadrant tenderness, and eosinophilia.[16] Heavy infections can 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. There does seem to be an anatomical preference for the left lobe of the liver that has been explained by small differences in the anatomy that cause these parasites to favor this portion of the liver.[7] Cholangiocarcinoma (bile duct carcinoma) is a long-standing sequelae due to chronic fibrosis and infection. It has a high mortality in Asia.

Diagnosis (see Clinical Appendix)

Four weeks after initial infection, eggs of these fish-borne trematodes start to be released

into human feces, so 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 may present for care, eggs may not be detected in the stool.[35] Flukes may be detected by endoscopic retrograde cholangiopancreatography (ERCP). The presence of flukes in the biliary tract may also be observed using ultrasound, CT, MRI and cholangiography.[36-40] A variety of serological tests, including Western blot and ELISA, are available, but cannot distinguish between current and past infection, and may cross react with other parasitic infections.[41, 42] NAATs (e.g., loop-mediated isothermal amplification (LAMP)) have been developed to improve the sensitivity of egg detection in stool, but 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 the treatment of *O. viverrini* and *O. felineus*.[48, 49]

Prevention and Control

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 together with spices and condiments to produce a dish roughly equivalent to ceviche. This concoction is a prime source of liver fluke infection. Thoroughly cooking contaminated fish and crustaceans is the most effective way of eliminating the parasite on an individual basis.[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, and in some areas the ingestion of raw fish has important roles in traditional cultural, and religious contexts.[7] There is also a long-held myth that hot spices and the consumption of alcohol 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 on a regular basis, 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 for the processing of human feces destined to be used as fertilizer.[50]

Molluscicides, alone, have not been used successfully for eradicating the intermediate snail hosts and there are concerns about the impact of their use on the environment.[51] The combination of regular draining of ponds, and molluscicides has been moderately effective in controlling infection in fish. Human vaccines are being tested and studied, and a vaccination strategy targeting the freshwater fish is being studied and tested as well.[52]

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35. *Fasciola hepatica*
(Linnaeus 1758)

Pronunciation: \fə-ˈsē-ə-lə\\hi-ˈpa-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, infecting wild animals and livestock of all kinds, and is endemic throughout Central America, the British Isles, southeastern United States, Africa, Europe (especially Turkey), Asia, the Middle East, and South America.[1, 2] Although less frequent in other parts of the world, cases have been reported in the United States and Australia.[3, 4] New Zealand also used to have *F. hepatica*, but aggressive eradication programs in the 1960s and '70s have eradicated it from that island country.[5] *F. hepatica* infects millions of people worldwide and has been reported in more than 50 countries.[6-9]

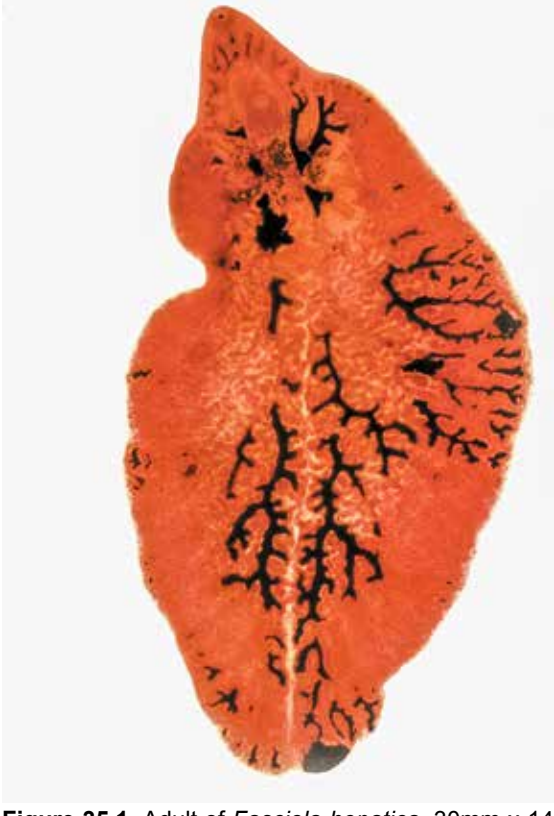


Figure 35.1. Adult of *Fasciola hepatica*. 30mm x 14 mm.

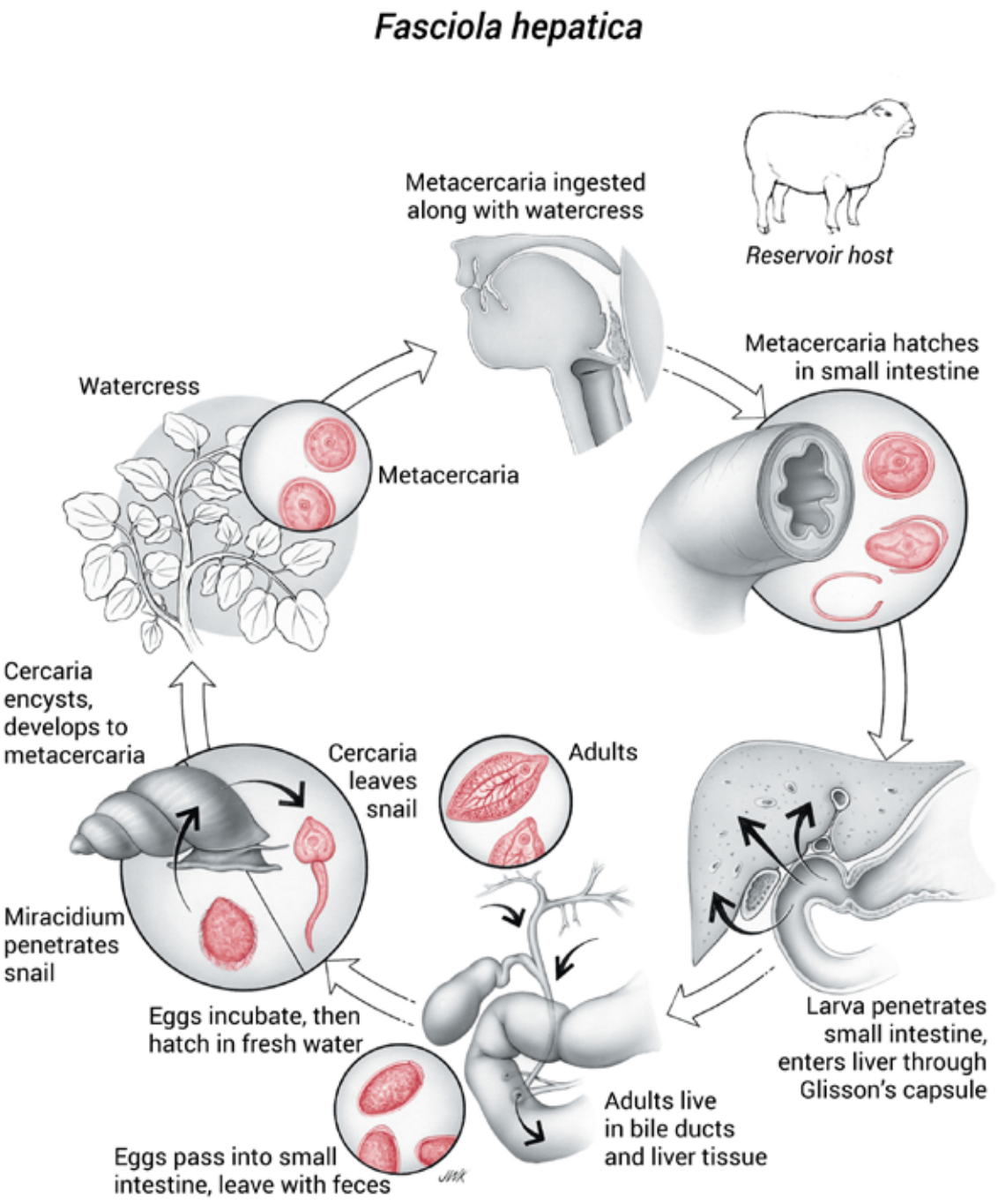
Intensity of infection in humans is always associated with animal husbandry.[10] In areas of South America, such as Peru and Bolivia, there is a high prevalence, particularly in the northwestern altiplano of Bolivia, near Lake Titicaca.[11] *Fasciola gigantica* is a closely related species infecting 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 in guinea pigs proving that the adult parasite was acquired by swallowing the infective stage.[22]

Life Cycle

Infection is initiated by ingestion of encysted metacercariae that are firmly attached to littoral vegetation, particularly watercress, in standing bodies of freshwater (e.g., farm ponds).[23] 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. Migrating metacercariae track through the liver causing necrosis and fibrosis. Only a small number reach the biliary tree to develop to sexual maturity.


Fasciola hepatica
Reservoir host
Metacercaria ingested along with watercress
Metacercaria hatches in small intestine
Watercress
Metacercaria
Cercaria encysts, develops to metacercaria
Cercaria leaves snail
Adults
Miracidium penetrates snail
Eggs incubate, then hatch in fresh water
Larva penetrates small intestine, enters liver through Glisson's capsule
Adults live in bile ducts and liver tissue
Eggs pass into small intestine, leave with feces

Maturation to reproductive adults takes up to 4 months. The adult fluke is large, measuring 35 mm by 15 mm (Fig. 35.1) and can live in the biliary tree for over a decade.

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 into the fecal mass. Eggs must be deposited in freshwater in order to embryonate, which may take as long as 9–15 days. The miracidium is stimulated to hatch by exposure to direct sunlight. After emerging from the egg, it is a free-swimming organism until it finds its snail host. The most common snail species for *F. hepatica* is *Lymnea truncatula* but many other species of Lymneid snails (e.g., *Fossaria modicella*) support the growth and development of this fluke throughout the world. The miracidium penetrates the snail's body wall, and finds its way to the hepatopancreas. It then undergoes sequential development, first into sporocysts, then into rediae, then into cercariae (Fig. 35.4) which emerge from the snail. These attach to the surfaces of littoral vegetation, where they become encysted. Within the cyst they transform into the environmentally resistant, infective stage, the metacercaria. This stage can live and remain infective for several months.

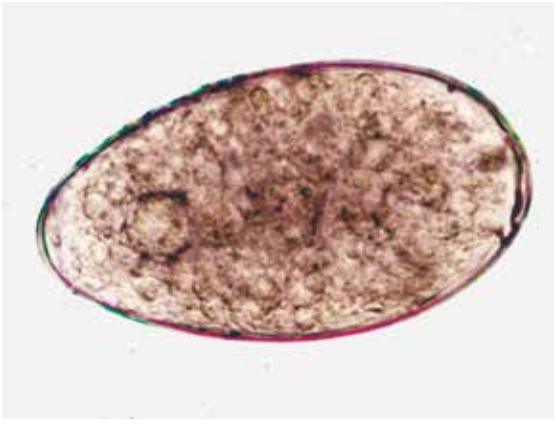


Figure 35.3. Egg of *F. hepatica*. 140 μm x 85 μm.

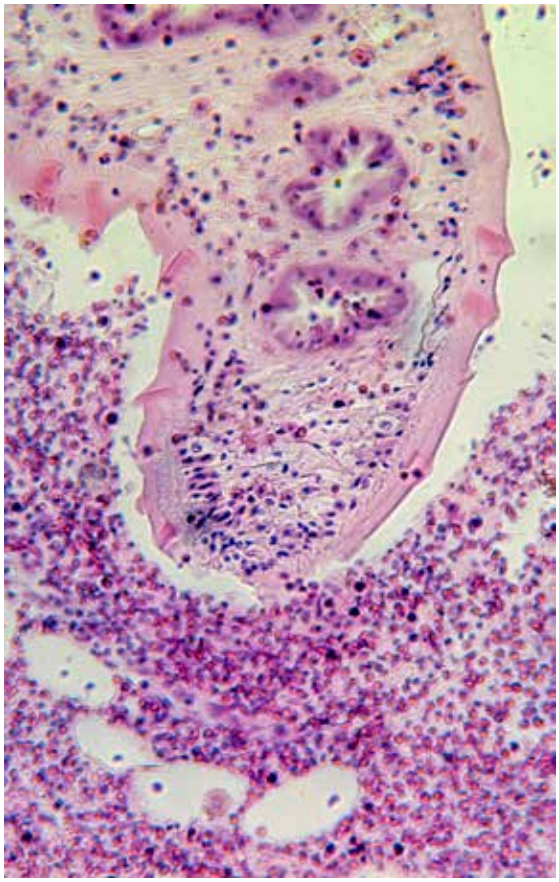


Figure 35.2. Histological section of adult *F. hepatica* in liver.

Ingested metacercariae sometimes find their way to tissues other than the liver (e.g., brain, kidney).[24, 25] In this case, they become aberrant infections and pass eggs that cannot find their way out of 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

Figure 35.4. Cercaria of *F. hepatica*. 100 μm.

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.

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 consumption of raw sheep liver that, although previously attributed to *F. hepatica,* 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 after becoming infected.[29] Many infected persons are asymptomatic during this early phase, while symptomatic patients may report fever, pain in the right upper quadrant of the abdomen, headache, generalized malaise, myalgia, weight loss, and urticaria.[32] 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 6 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 it may 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. It is only after enough time has gone by (up to 4 months) that mature adults, present in the biliary tree, release eggs into the stool. During acute infection, serology usually becomes positive while the juvenile worms are migrating through the liver parenchyma. Most patients will present at this stage with high levels of circulating eosinophils.[29] Serological tests can be useful in ruling in the diagnosis at this stage of the infection, and have excellent sensitivity and specificity.[36-38] *F. hepatica* circulating antigen

tests, also with excellent sensitivity and specificity, are available and correlate with 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, in bile aspirates, or in 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 making the diagnosis of 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 treatment of 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. Praziquantel, which is excellent for treating infections with other flukes, is not effective against Fasciola, nor are mebendazole, albendazole, or artesunate.[49-51] Nitazoxanide appears to be an inferior but alternate therapy with some demonstrated efficacy.[52, 53] Successfully treated patients will develop negative serologies 6–12 months after clearing their parasites.

Prevention and Control

Fasciola 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] When infections are detected, appropriate treatment in both groups is warranted. 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 animal use that have demonstrated some degree of efficacy in terms of reduction in egg production and worm burdens.[54] Despite significant advances in vaccine development, no human or animal vaccine is in current use.[55]

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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-ˈgän-ə-məs\ \west-ər-mən-ī\

Paragonimus kellicotti
(Ward 1908)

Pronunciation: \par-ə-ˈgän-ə-məs\ \kel-i-kot-ī\

Introduction

There are more than 40 species in the genus *Paragonimus* (\par-ah-GON-i-mus\), but 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*. While most members of this genus are relatively restricted in distribution, *P. westermani* is widely found throughout the world.[11] *P. westermani* will be presented as the model organism for food-borne lung flukes affecting humans, but *P. kellicotti*, which is indigenous to the United States, and certain other *Paragonimus* spp. will be mentioned.[12-14]

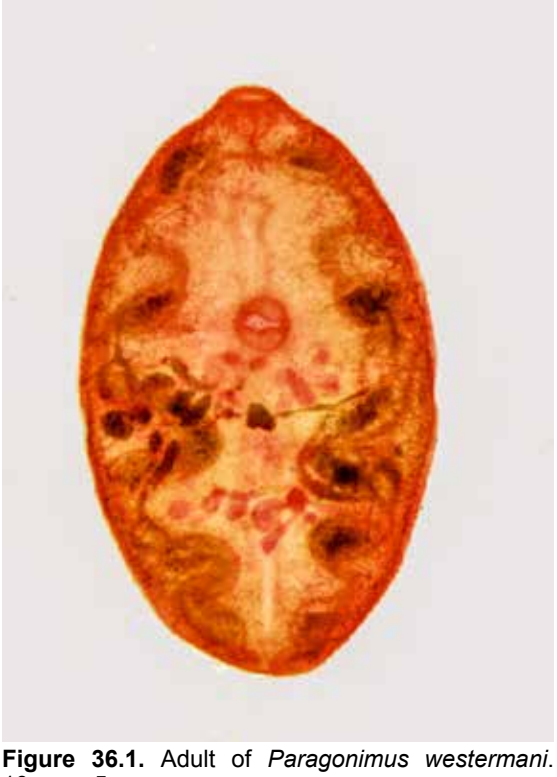


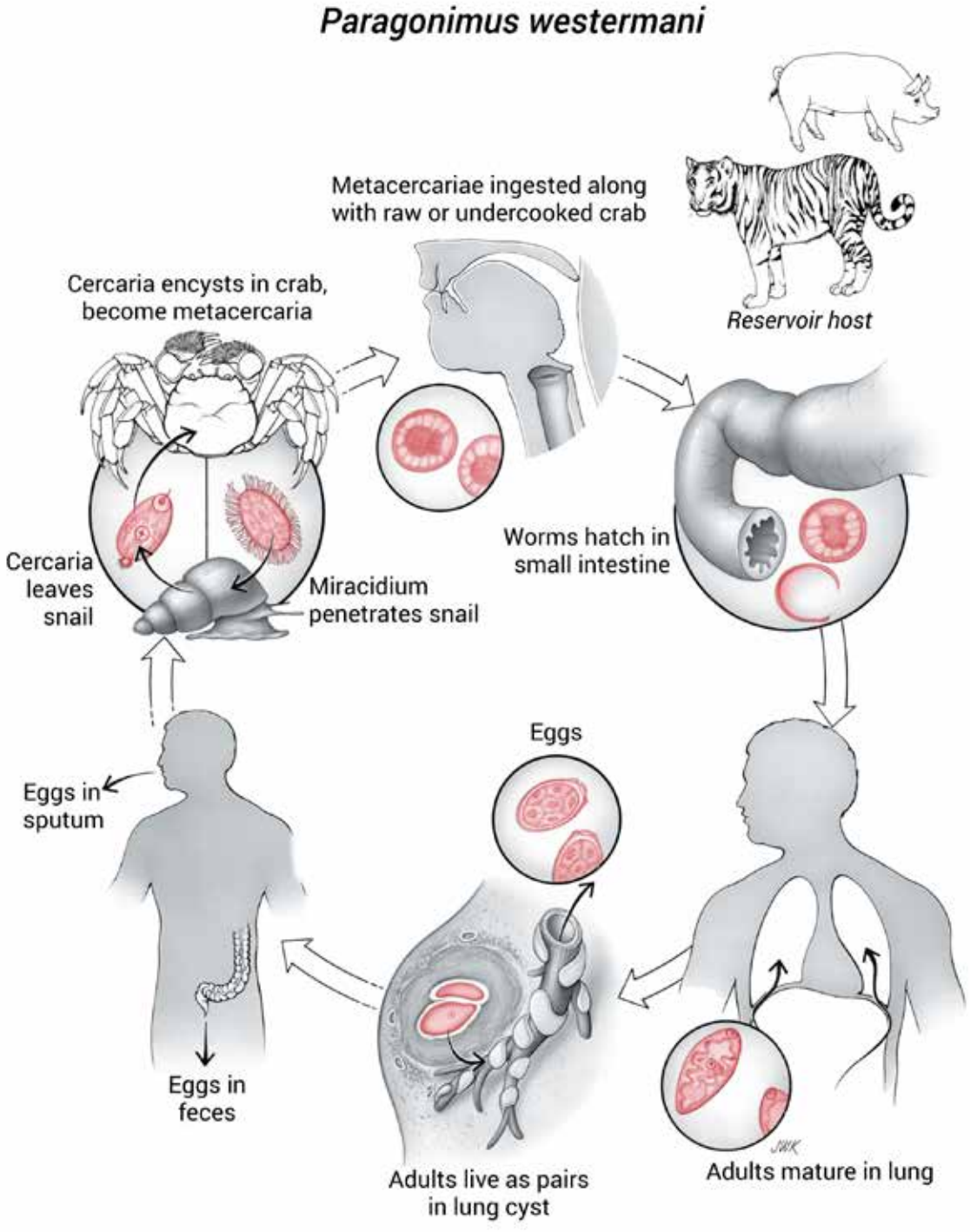
Figure 36.1. Adult of *Paragonimus westermani*. 10mm x 5 mm.

Although hermaphroditic, most *Paragonimus* spp. do not self-fertilize. Instead, they live typically as 2 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 a wide range of reservoir hosts, including fox, civet, tiger, leopard, panther, mongoose, wolf, pig, dog, and cat. It employs numerous crustaceans as intermediate hosts and that is what accounts 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. kellicoti*, a lung fluke of mink and opossums in the United States, has also caused infection in humans.[12]

Historical Information

In 1878, Coenraad Kerbert described the adult worm that he isolated at autopsy from a Bengal tiger.[19] In 1916, Koan Nakagawa implicated the freshwater crab as the intermediate host in the transmission of *P. westermani*.[20] In 1915, Sadamu Yokagawa deciphered the correct route of migration 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


Paragonimus westermani
Metacercariae ingested along with raw or undercooked crab
Reservoir host
Cercaria encysts in crab, become metacercaria
Worms hatch in small intestine
Cercaria leaves snail
Miracidium penetrates snail
Eggs
Eggs in sputum
Eggs in feces
Adults live as pairs in lung cyst
Adults mature in lung

reported on most of the clinical features of the disease, and also identified eggs of *P. westermani* in the sputum of patients with hemoptysis.[22, 23] 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 2 or more worms. More than 50 species of crustaceans are able to 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), that excyst in the small intestine.[13] Metacercariae penetrate into the abdominal cavity, and within several days, develop to immature flukes. The worms migrate to the lungs by penetrating the diaphragm and mature to reproductive adults within 8–12 weeks (Fig. 36.3). Worms also locate to aberrant sites, including brain, liver, intestines, muscle, skin, and testes. In these sites, passage of eggs to the external environment is not possible.

Figure 36.3. Histological section of adult *P. westermani* in lung.

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 production begins about 30 days after ingestion

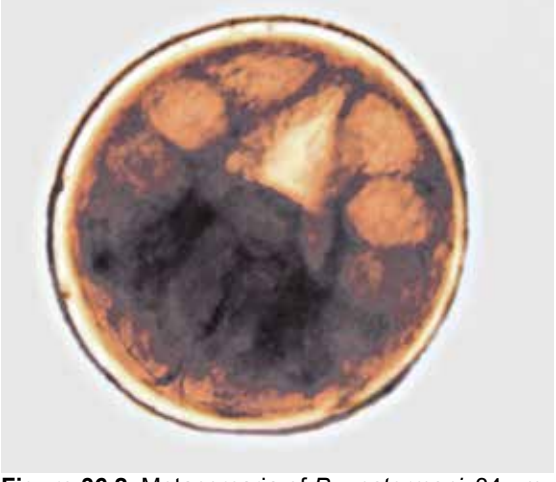


Figure 36.2. Metacercaria of *P. westermani*. 34 μm.

Figure 36.4. Egg of *P. westermani*. 110 μm x 60 μm.

of the metacercariae. Eggs (Fig. 36.4) pass fertilized, but unembryonated, into the surrounding tissue. Eventually, they reach the bronchioles and are included into the sputum that also contains blood and debris from the necrotic lesions created by the adults. Because some of the sputum is swallowed, eggs can be recovered from feces as well as sputum. The eggs must reach freshwater to embryonate. The miracidium develops over a 3-week period, after which it hatches and seeks out its intermediate snail host (e.g., *Melania* spp., *Semisulcospira* spp., and *Thiara* spp.). In contrast to other trematodes such as *Schistosoma* spp., *Paragonimus* spp. are able to mature in a large variety of 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]

The inflammatory responses to *Paragonimus* cysts are characterized by a variety of cells, but eosinophils usually predominate. Charcot-Leyden crystals can frequently be found in the sputum of infected individuals. Specific IgG and IgE antibodies are produced 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 time of ingestion of infective metacercariae and lasts up until flukes mature into egg-producing adults. During this stage of infection some patients may remain asymptomatic, 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 subcutaneous swellings that are migratory.[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, there may be pneumothorax and pleural effusion, with consequent pleural adhesions.

An often-fatal clinical outcome results from extrapulmonary paragonimiasis. Immature flukes may migrate to a number of 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] A number of cases of acid-fast bacilli-negative pulmonary infections are due to paragonimiasis. One distinguishing feature is the presence of Charcot-Leyden crystals on histopathology.[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 use of a 10x rather than the standard 100x objective employed in the diagnosis of mycobacterial infections.[38, 39]

If sputum or other body fluids, such as CSF or pleural fluid, is negative for eggs on repeated sampling, indirect evidence of infection can be obtained by use of serological tests, such as ELISA and Western blot.[41-43] These tests are particularly helpful in early stage disease as well as extrapulmonary disease. 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. kellicotti* infection in the U.S..[25]

Antigen detection tests have been developed, but are not routinely used in clinical practice.[44] NAATs are being developed that allow for species identification.[45] There is also a simple and rapid intradermal test, 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] Both the serologic and intradermal assays indicate either current or past exposure to the infection.

Imaging tests such as CT and fluorodeoxyglucose-positron emission tomography (FDG-PET) can help with the diagnosis 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 who has 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)

The drug of choice against *Paragonimus* spp. is praziquantel, while triclabendazole is an alternative drug with similar efficacy.[24, 50-54] Another regimen that has been 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 its worldwide distribution, many consider control of this parasite in animals to be impractical in most parts of the world.[13] Due to cultural eating habits favoring the acquisition of this parasite, control of paragonimus infection is difficult, and would require a comprehensive approach.[56] For example, "drunken hairy crab" is traditionally eaten live, and in the modern city of Shanghai, its 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 the control of paragonimiasis.

In certain endemic regions of the world, mass chemotherapy has been attempted to reduce prevalence rates.[57-60] Boiling the invertebrate host for several minutes until the meat has congealed and turned opaque can kill the metacercariae. Marinating and salting of crabs or other crustaceans does not reliably kill the infective stages.[24]

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37. Other Trematodes of Medical Importance

Besides the trematode infections already identified as major causes of human disease throughout the world, other trematode species continue to have a negative impact on the human condition, but not quite to the extent of schistosomiasis, for example. A few of these "rare" infections are actually not so rare in some geographic regions, and deserve more than a mention, since cases of exotic infections are becoming more common in western clinics due to increased immigration from those regions. Many of them are zoonotic and classify 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 major 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 commercial 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, ovoid, 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,

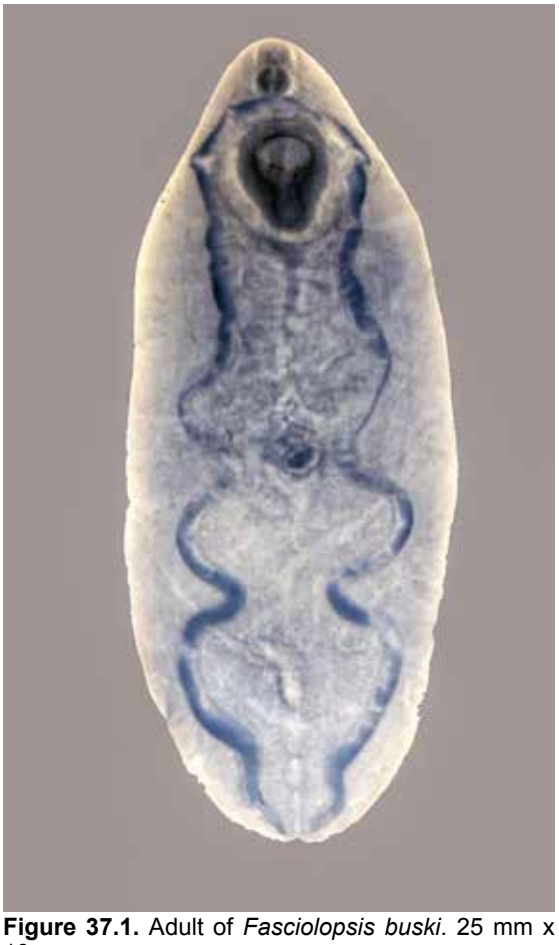
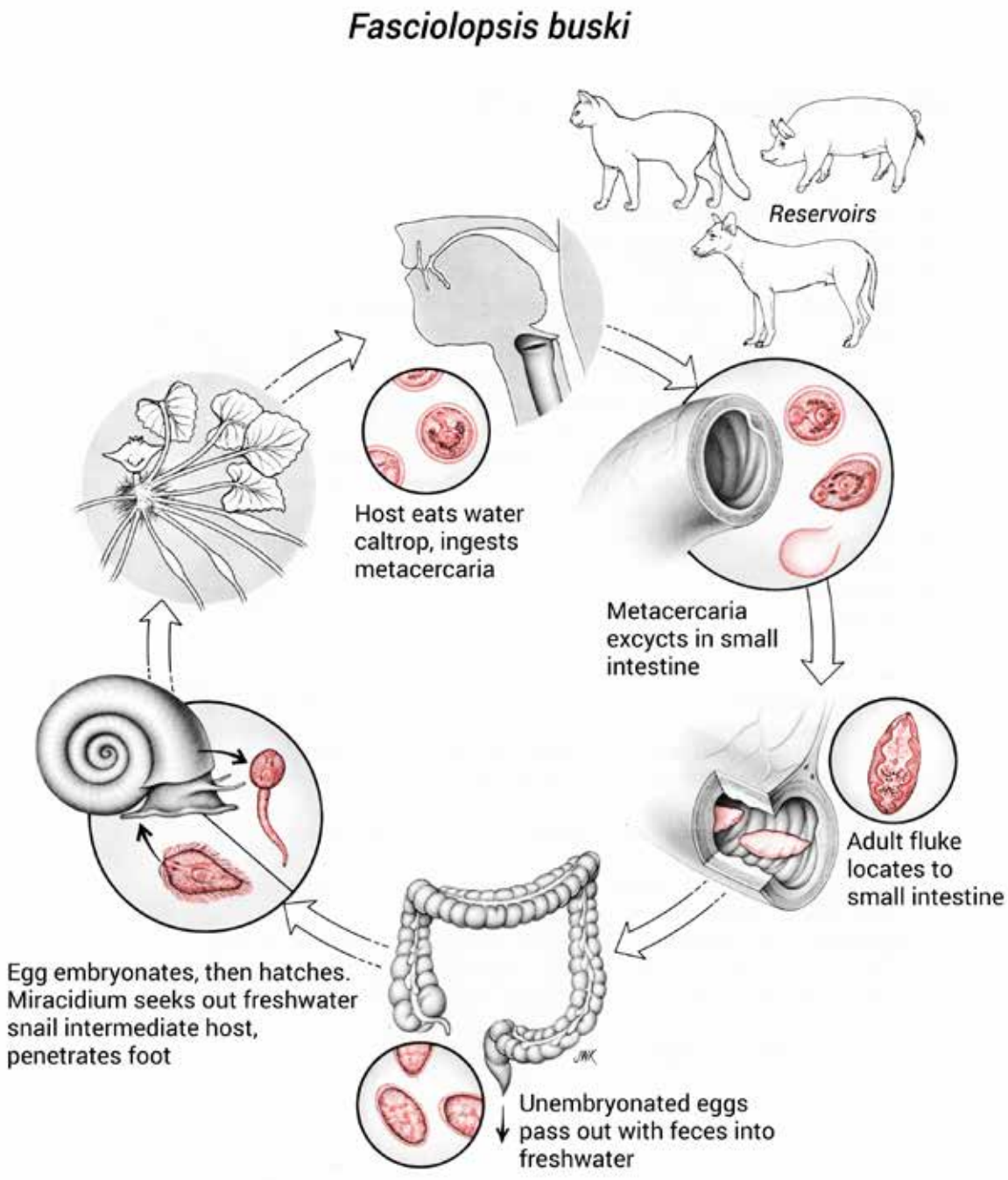


Figure 37.1. Adult of *Fasciolopsis buski*. 25 mm x 10mm.


Fasciolopsis buski
Reservoirs
Host eats water caltrop, ingests metacercaria
Metacercaria excycts in small intestine
Adult fluke locates to small intestine
Egg embryonates, then hatches. Miracidium seeks out freshwater snail intermediate host, penetrates foot
Unembryonated eggs pass out with feces into freshwater

and finally into cercariae. After leaving the snail, the cercariae swim about and come to rest on littoral 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 hemorrhage, 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] An elevated level of circulating eosinophils is a common feature of even light infection with *F. buski*.

Diagnosis

Definitive diagnosis is by 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. For travelers to endemic areas one should make sure that all aquatic plants are well cooked prior to ingestion. Reservoir hosts apparently play a role in the maintenance of this parasite in endemic regions, and consequently it is recommended that raw

Figure 37.2. Egg of *F. buski*. 140 μm x 80 μm.

aquatic plants are not fed to pigs.[13] The habit of shucking water chestnuts by placing 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 that of *Fasciola*, except that the metacercariae encyst in various species of snails, tadpoles, or freshwater fish.[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 has been through identification of the flukes on endoscopy.[18] Cur-

rently 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-ˌrōˈfī-ēz\\he-tə-ˌrōˈfī-ēz\

Metagonimus yokogawai
(Katsurada 1912)

Pronunciation: \me-tə-gòn-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 little damage there. *H. heterophyes* is found throughout Asia, the Middle East, and Africa. *M. yokogawai* is also common in Asia, but foci of infections have been reported in Spain and Russia. A few human infections with *Heterophyes nocens*, a related species, have been reported from Korea.[19]

Figure 37.3. Adult of *Heterophyes heterophyes*. 2 mm x 0.5 mm.

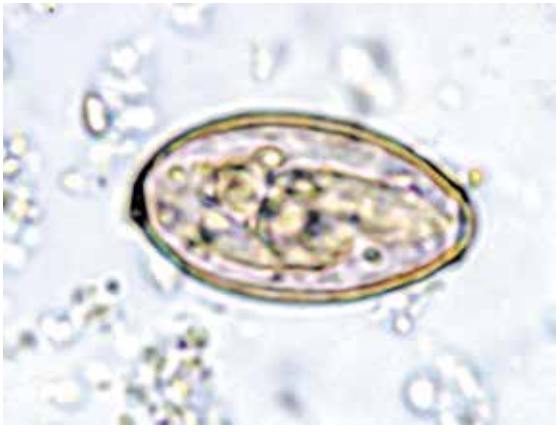


Figure 37.4. Egg of *H. heterophyes*. 25 μm x 13 μm.

Life Cycles

Infection begins with the ingestion of 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 thereafter. The fully-embryonated eggs pass out with the fecal mass into brackish or freshwater.

H. heterophyes primarily infects snails of the genus *Cerithidia*, while those of *M. yokogawai* 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 of these parasites varies widely with 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.

Figure 37.6. Egg of *M. yokogawai*. 25 μm x 15 μm.

Clinical Disease

Like other trematode infections, the clinical presentation is thought to be determined in 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]

Figure 37.5. An adult of *Metagonimus yokogawai*. 2.5 mm x 0.6 mm.

Diagnosis (see Clinical Appendix)

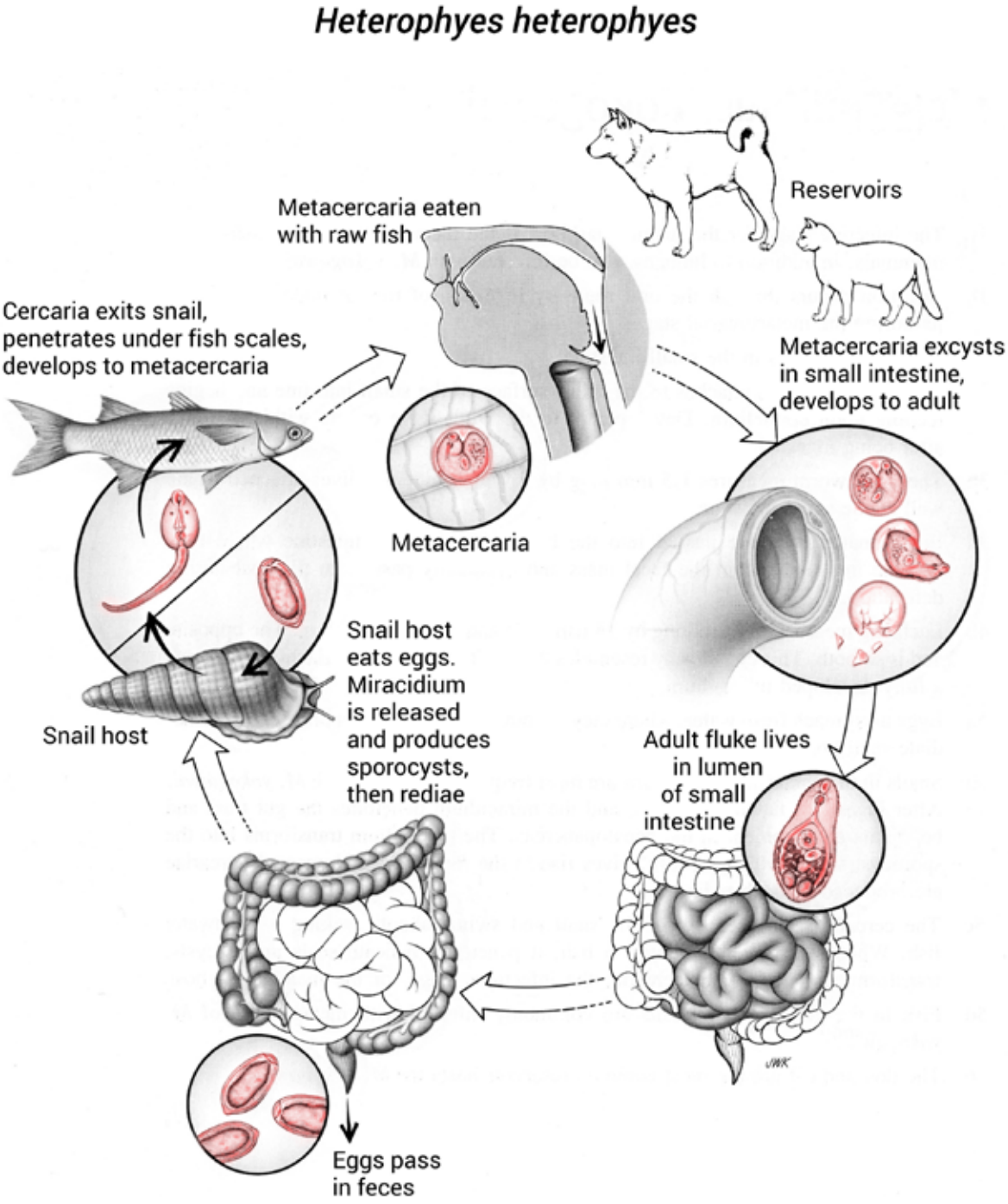
Diagnosis is usually based on recovery and identification of eggs in feces. The eggs (Figs. 37.5, 37.6) of *H. heterophyes* and *M. yokogawai* closely resemble those of *C. sinensis* (Fig. 34.4). They must be carefully differentiated by their absence of a terminal knob and a collar at the operculum. Eggs and even adult flukes have been recovered on endoscopy.[18, 23, 24]

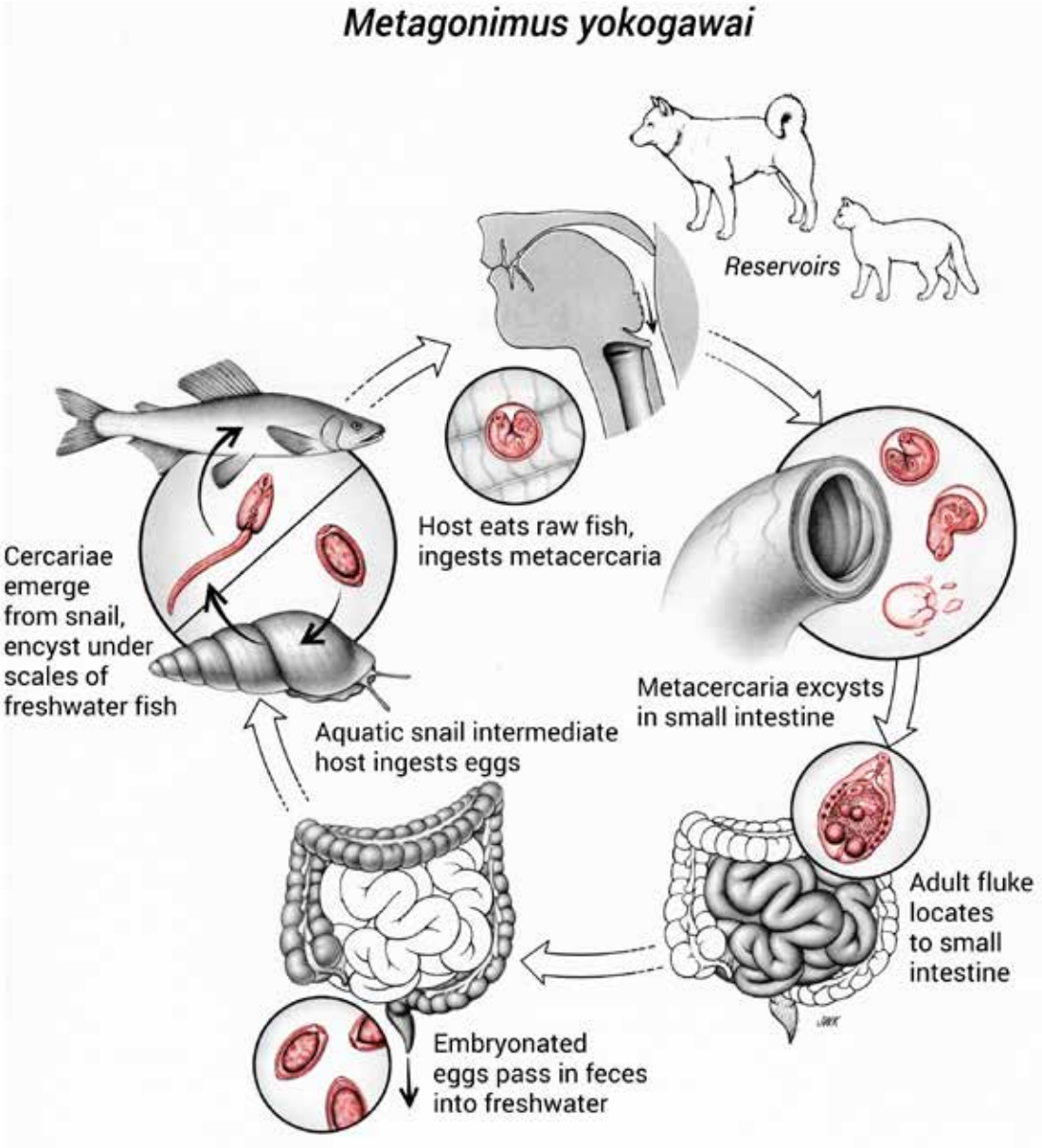
Treatment (see Clinical Appendix)

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 by controlling the indiscriminate use of untreated human feces as fertilizer.[1, 17, 20] Protection of fish ponds from contamination with human feces and control of snail populations are potentially helpful.[14]


Heterophyes heterophyes
Reservoirs
Metacercaria eaten with raw fish
Cercaria exits snail, penetrates under fish scales, develops to metacercaria
Metacercaria excysts in small intestine, develops to adult
Metacercaria
Snail host eats eggs. Miracidium is released and produces sporocysts, then rediae
Snail host
Adult fluke lives in lumen of small intestine
Eggs pass in feces


Metagonimus yokogawai
Reservoirs
Host eats raw fish,
ingests metacercaria
Cercariae
emerge
from snail,
encyst under
scales of
freshwater fish
Metacercaria excysts
in small intestine
Aquatic snail intermediate
host ingests eggs
Adult fluke
locates
to small
intestine
Embryonated
eggs pass in feces
into freshwater

Nanophyetus salmincola
(Chapin 1927)

Pronunciation: \nānəˌfīˈētəs\\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 tends to mostly infect animals, and only 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 poisoning" 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, along with a history of ingestion of raw or poorly cooked salmon.[28] Praziquantel is the recommended treatment but niclosamide may also be efficacious.[28]

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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

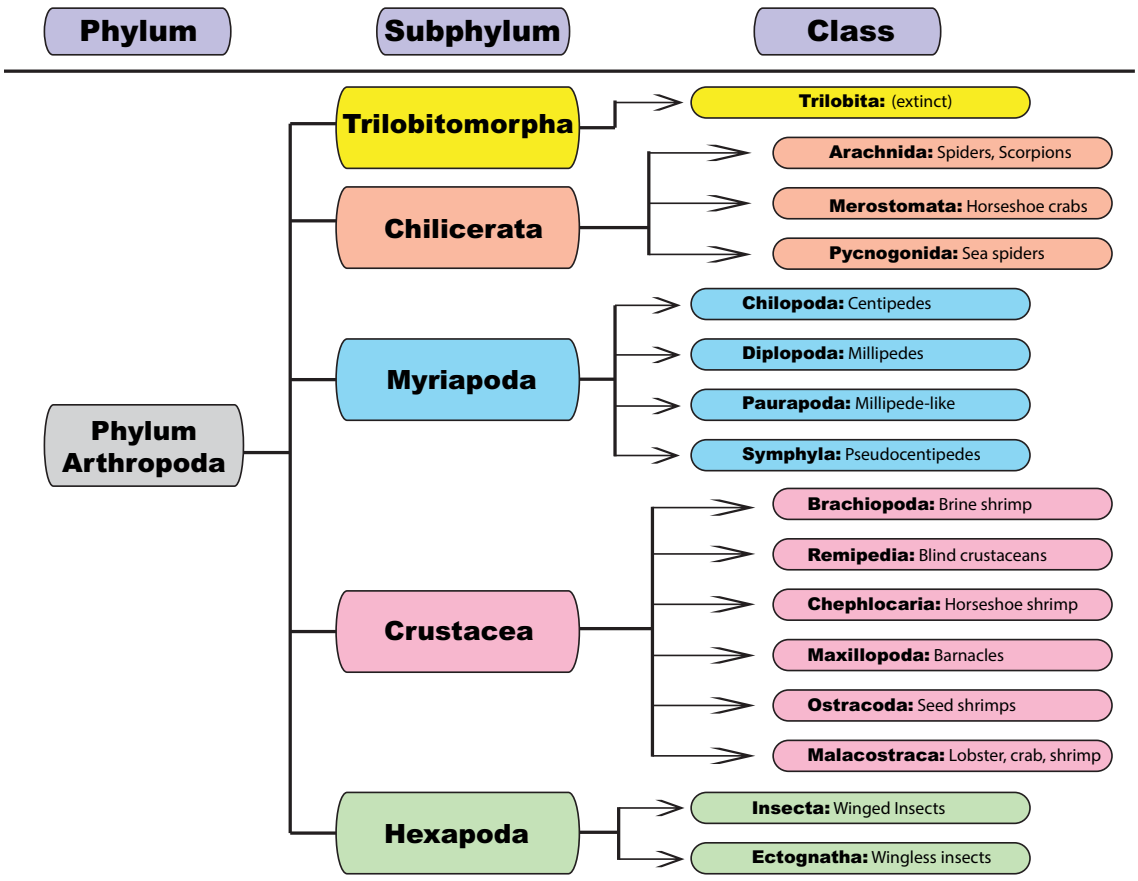
Arthropods directly influence humans' well-being, not only because they are hosts of parasitic organisms and vectors of a wide variety of pathogens, but also by causing tissue damage and disease. They also affect human health by reducing the availability of food. Insects are essential pollinators of 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, entomophobia 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.

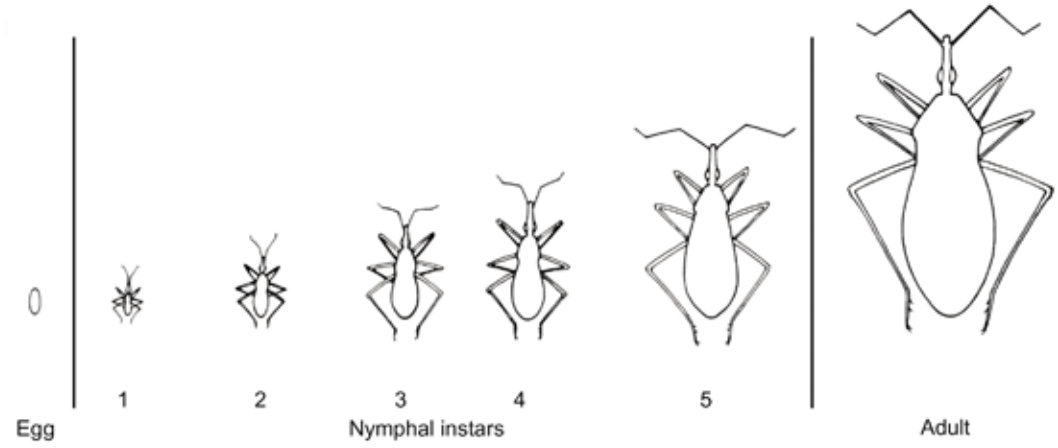


Figure 38.1. Incomplete metamorphosis in insects. Typical example of incomplete metamorphosis is the kissing bug, *Rhodnius prolixus*. Immature stages are wingless, smaller versions of the winged adult. All stages, except the egg, have three pairs of legs. The number of nymphal stages varies with the species.

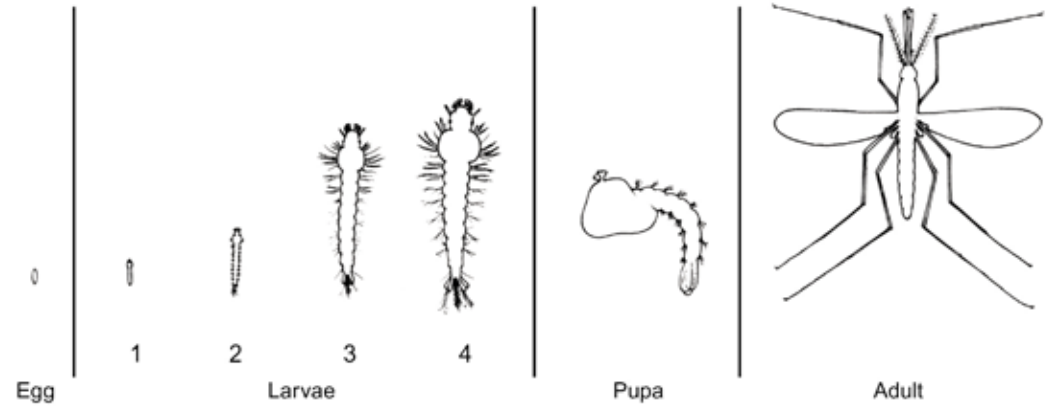


Figure 38.2. Complete metamorphosis in an insect with aquatic immature stages. The *Anopheles* mosquito begins as an egg laid on the surface of water and develops through four larval stages and a single pupal stage to a sexually mature, winged adult.

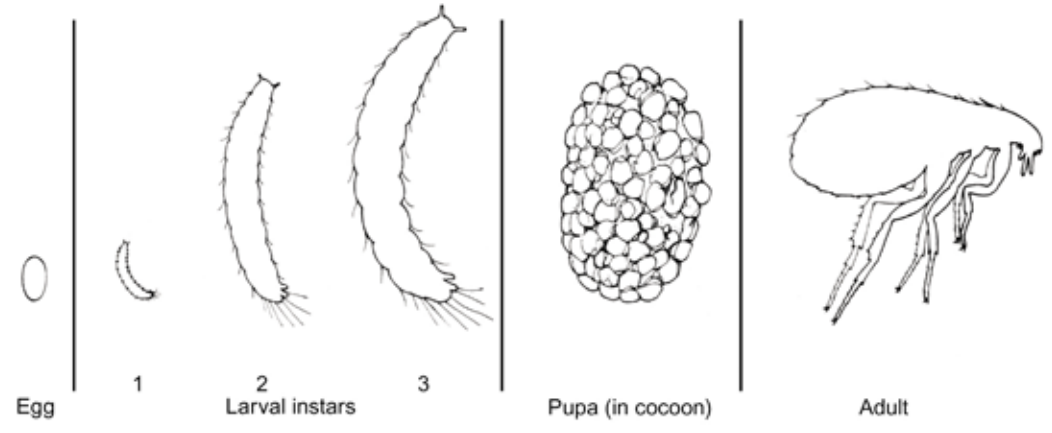


Figure 38.3. Complete metamorphosis in an insect with terrestrial immature stages. The flea begins as an egg laid on the fur of the host or in the nest area. Several maggot-like larval stages are followed by a single pupal stage,encased in a sand-covered cocoon, from which a wingless, sexually mature 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)			
Pediculus humanus humanus	Body louse	Worldwide	Skin reactions to bites, vectors of rickettsiae and spirochetes
P. humanus capitis	Head louse	Worldwide	Skin reaction to bites
Phthirus pubis	Crab louse	Worldwide	Skin reaction to bites
Heteroptera (true bugs)			
Cimex lectularius	Bed bug	Worldwide	Skin reaction to bites
C. hemipterus	Tropical bed bug	Tropical and subtropical	Skin reaction to bites
Triatoma infestans *Rhodnius prolixus* *Panstrongylus megistus*	Kissing bug, cone-nosed bug	Tropical and subtropical regions of the New World	Skin reaction to bites, vectors of *Trypanosoma cruzi* (cause of Chagas disease)
Hymenoptera (bees, wasps, ants)			
Apis mellifera	Honey bee	Worldwide	Painful sting, potential anaphylaxis
Bombus 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
Solenopsis spp.	Fire ant	Tropical America, southeastern United States	Painful bite and multiple stings, potential anaphylaxis
Diptera (flies, mosquitoes, and their relatives)			
Ceratopogonidae *Culicoides* spp. *Leptoconops* spp.	No-see-um, sand fly	Worldwide	Serious biting pest, skin reaction to bites, vectors of several filariid nematodes
Psychodidae *Phlebotomus* spp. *Lutzomyia* spp.	sand fly	Worldwide	Skin reaction to bites, vectors of Leishmania, Pappataci fever, and Carrion's disease
Simuliidae *Simulium* spp.	Black fly, buffalo gnat	Worldwide	Serious biting pests, skin reaction to bites, vectors of Onchocerca and Mansonella
Culicidae *Aedes* spp. *Anopheles* spp. *Culex* spp. *Culiseta* spp. *Mansonia* spp.	Mosquito	Worldwide	Serious biting pests, skin reaction to bites, vectors of viruses, protozoa, and filaria
Tabanidae *Tabanus* spp. *Chrysops* spp.	 Horsefly Deerfly	 Worldwide Worldwide	 Biting pests, painful bite followed by skin reaction Biting pests, vectors of tularemia and *Loa loa*.
Muscidae *Musca domestica* *Stomoxys calcitrans*	 House fly Stable fly	 Worldwide Worldwide	 Mechanical disseminator of pathogens Serious biting pest
Glossinidae *Glossina* 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)			
Xenopsylla cheopis *Ctenocephalides felis* *C. canis* *Pulex irritans* *Tunga penetrans*	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 punkies, 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 Sergent 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 anthropophilic; they feed on dogs and rodents as well.

Figure 38.4. Adult female phlebotomine sand fly, *Lutzomyia anthophora.* Courtesy J. Ribeiro.

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 *verruga 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 papatasii* 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 mosquitos 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 38.7. *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 38.6. *Anopheles dirus*, one of the major malaria vectors in Southeast Asia, performing "plasmapheresis".

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-

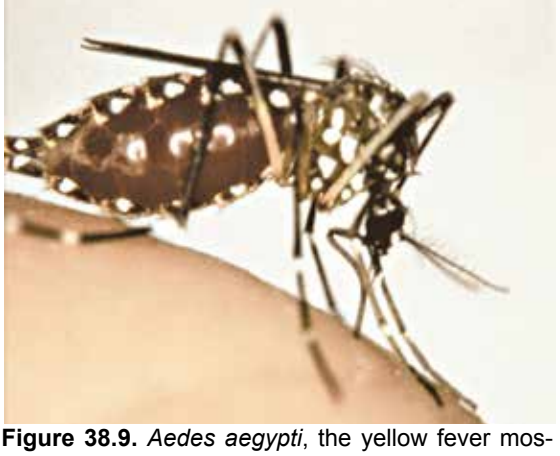


Figure 38.9. *Aedes aegypti*, the yellow fever mosquito, in a typical feeding position.

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 "breakbone 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 mosquitoes, 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. tritaniorhynchus, 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 repellant 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-ˌäps\) 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.

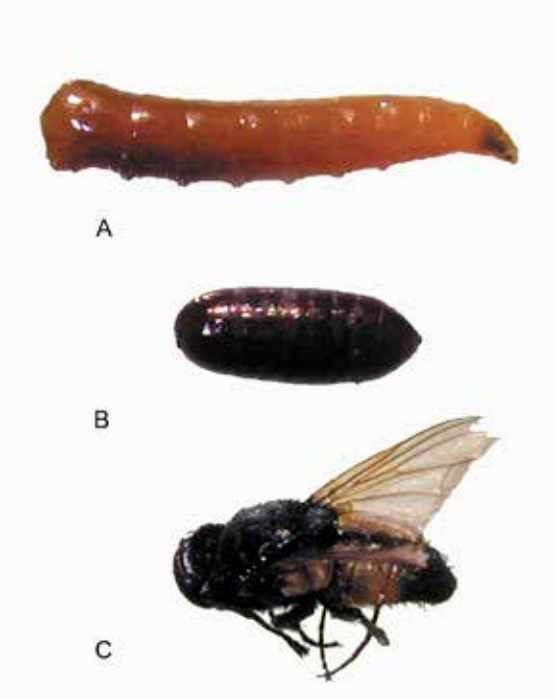


Figure 38.10. *Musca domestica*, the housefly. A. Larva. B. Pupa. C. Adult. The larvae of flies are referred to as maggots.

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 repellants 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*.

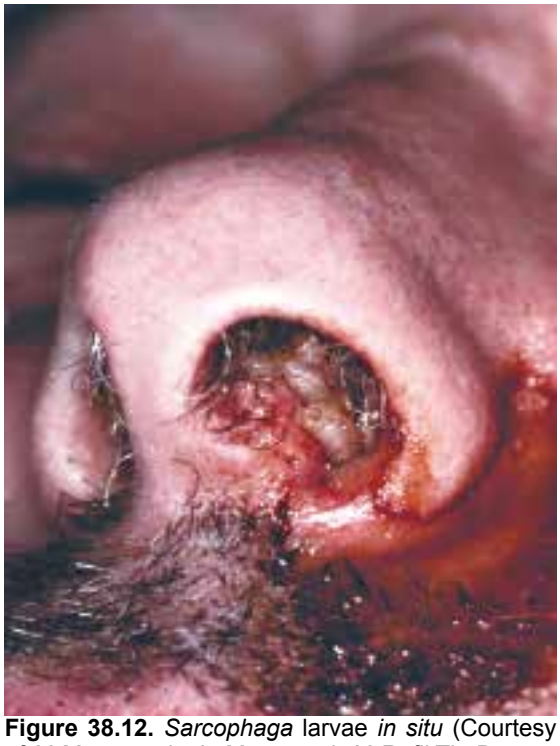


Figure 38.12. *Sarcophaga* larvae *in situ* (Courtesy of Y. Mumcouglu. In Mumcouglu Y, Rufli Th: Dermatologische Entomologie. Perimed Fachbuch, Erlangen, 1982).

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]

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

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]

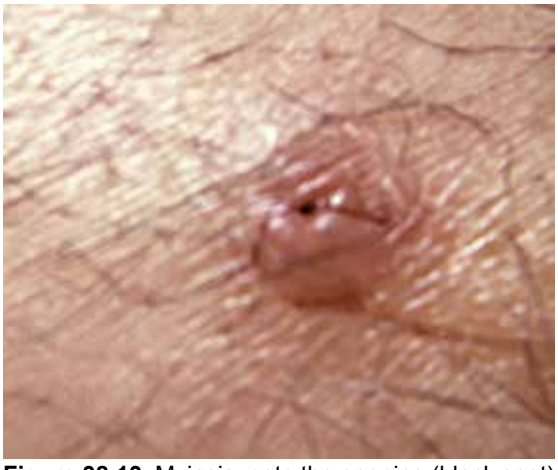


Figure 38.13. Myiasis: note the opening (black spot) in the skin which permits the maggot, burrowing in the tissue below, to breathe.

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

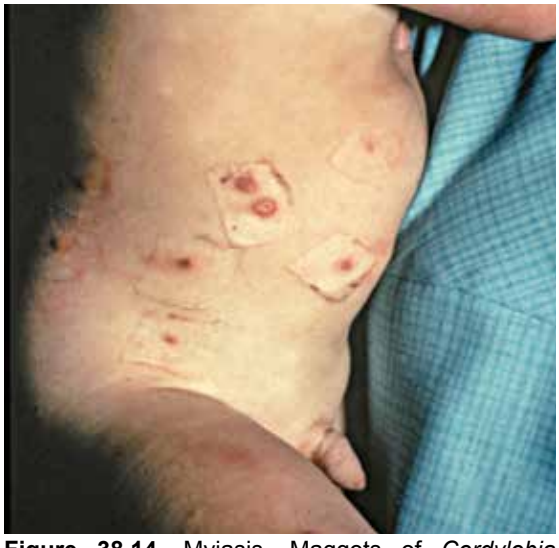


Figure 38.14. Myiasis. Maggots of *Cordylobia anthropophaga* in the flesh of an infant. Note the raised areas and openings for the larvae to breath.

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 penetrate 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. Screwworms 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

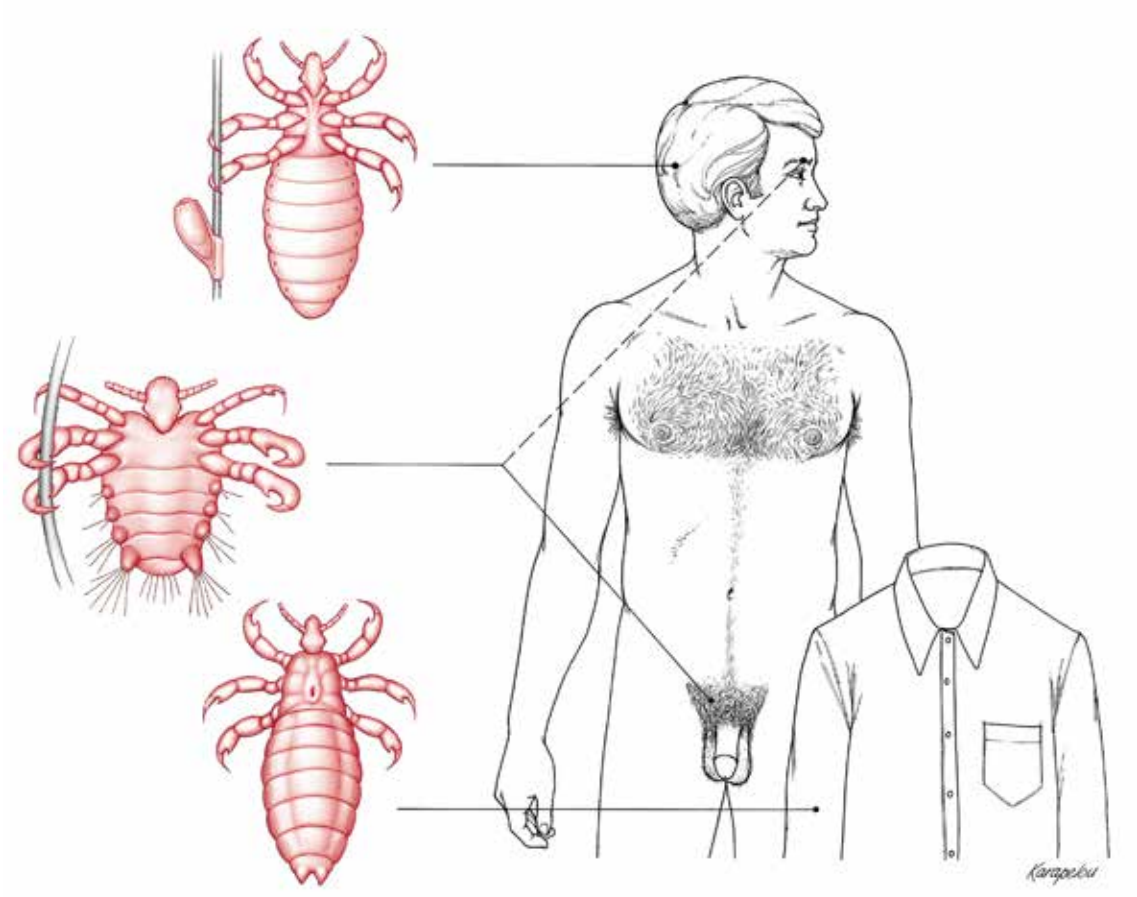


Figure 38.17. Preferred feeding and resting sites of the three species of louse affecting humans. *Pediculus humanus capitis*, the head louse, resides, feeds, and reproduces on the hairs of the head. Eggs are laid individually on hair shafts. *Phthirus pubis*, the crab louse, prefers hair of the pubic 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.

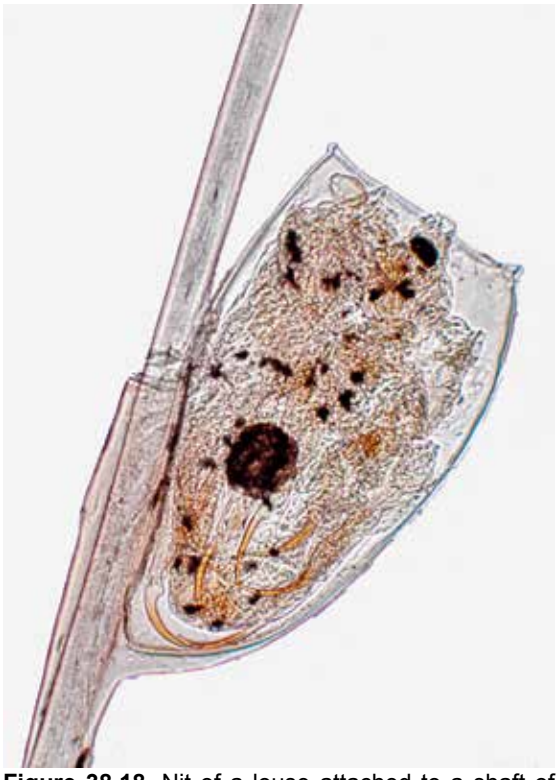


Figure 38.18. Nit of a louse attached to a shaft of hair.

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 lice as disease vectors is more recent. Transmission of typhus and relapsing fever by lice was not demonstrated until the early 1900s.

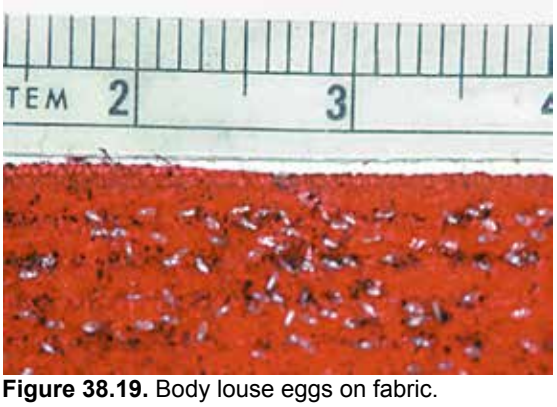


Figure 38.19. Body louse eggs on fabric.

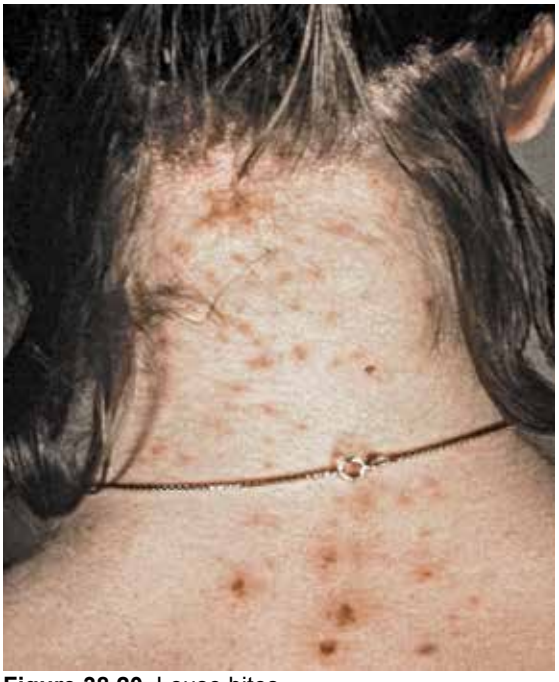


Figure 38.20. Louse bites.

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 body lice. Infestations with head lice can reach epidemic proportions, particularly among schoolchildren.[67, 68]

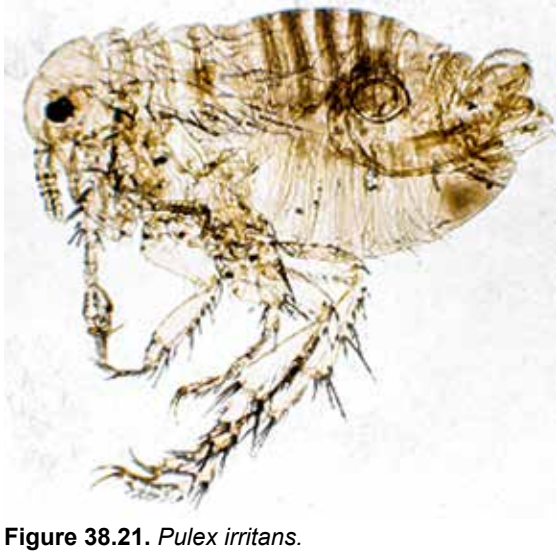


Figure 38.21. *Pulex irritans.*

Figure 38.22. *Xenopsylla cheopis*, an important vector of plague.

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 prowazeki*, 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.

Figure 38.24. *Tunga penetrans* in skin. Courtesy G. Zalar.

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 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.

Figure 38.23. *Ctenocephalides felis.* Photo D. Scharf.

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 species, 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.

Figure 38.26. Bed bug adult. *Cimex lectularius* 15x.

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

manifestation of flea bites. The major health problem caused by fleas is the transmission of infectious agents for which the fleas are vectors.

Diagnosis

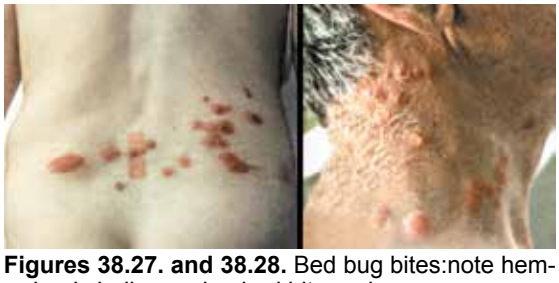
The typical fleabite first appears as a single papule. With heavy flea infestations, papules may be grouped along the arms and legs, on the face and neck, or where clothing fits snugly. Precise incrimination of fleas requires capture of one of the offending insects. The species of the flea can be determined 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



Figures 38.27. and 38.28. Bed bug bites:note hemorrhagic bullae and paired bitemarks.

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

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-ˈmip-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 Climicidae 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).

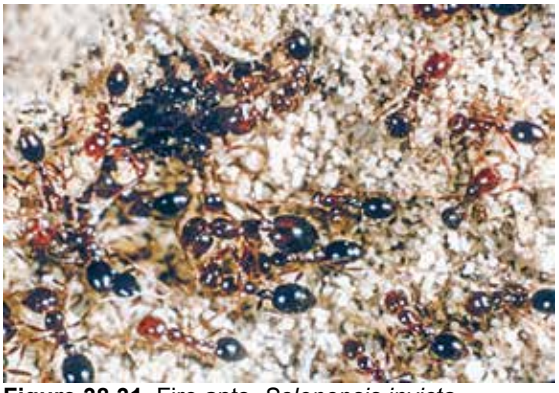


Figure 38.31. Fire ants, *Solenopsis invicta*.

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 µl of blood, and adult females may ingest more than 200 µl 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*, originally 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 resmithrin (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.

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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.

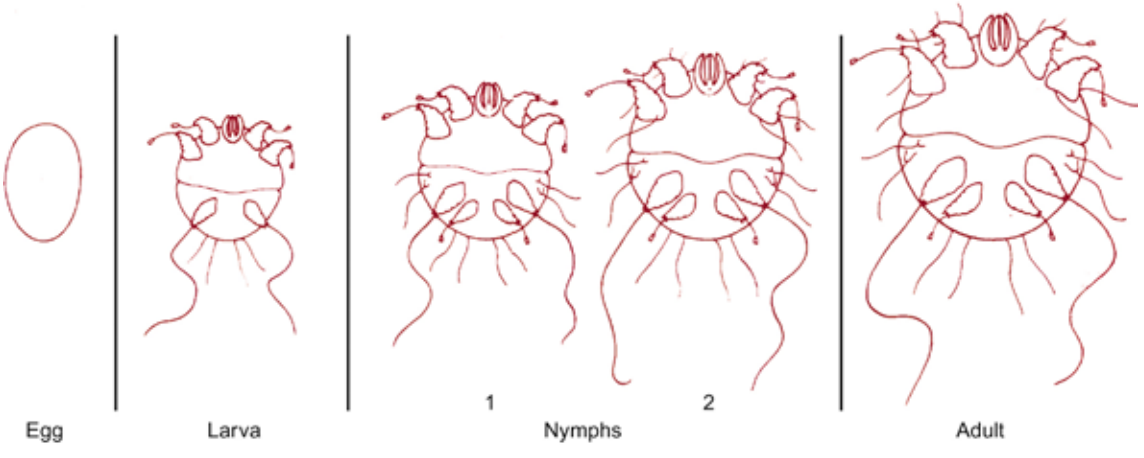

Figure 39.1. Incomplete metamorphosis in the arachnida. Ticks and mites undergo incomplete metamorhosis as typified by the itch mite, *Sarcoptes scabiei*. Larvae have three pair of legs; adults and nymphal stages have four pairs of legs.

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 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]

Figure 39.2. Deer tick, *Ixodes scapularis*. Photo D. Scharf.

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.

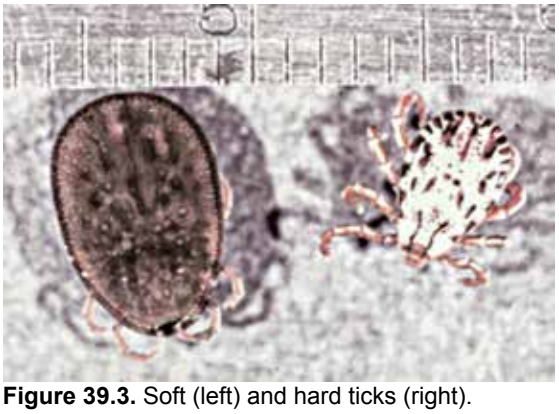

Figure 39.3. Soft (left) and hard ticks (right).

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 *Liponyssoides sanguines*	House mouse mite	Worldwide	Vector of *Rickettsia akari*, the cause of rickettsial pox
Various other genera	Mite	Worldwide	Occasional dermatitis from bite
Demodicidae *Demodex folliculorum*	Follicle mite	Worldwide	Found in sebaceous glands and hair follicles Occasional skin reactions
Trombiculidae *Trombicula* spp. *Leptotrombidium palpale*	Harvest mite Mite	Worldwide Southeast Asia, India, Pacific Islands	Intense itching at site of attachment Vector of *Orientia tsutsugamushi*, cause of scrub typhus
Sarcoptiae *Sarcoptes scabiei*	Human itch mite	Worldwide	Burrows in skin causing severe itching
Araneae (spiders)			
Latrodectus spp. *Loxoceles* 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 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 39.5. *Ixodes scapularis*. The adult feeds on deer. Nymphs transmit spirochetes and *Babesia* to humans.

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 several 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 *Ornithodorus* 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 *Ornithodorus* 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 *Ornithodorus* 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 *Franciscella 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 hemocele, 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 function 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]

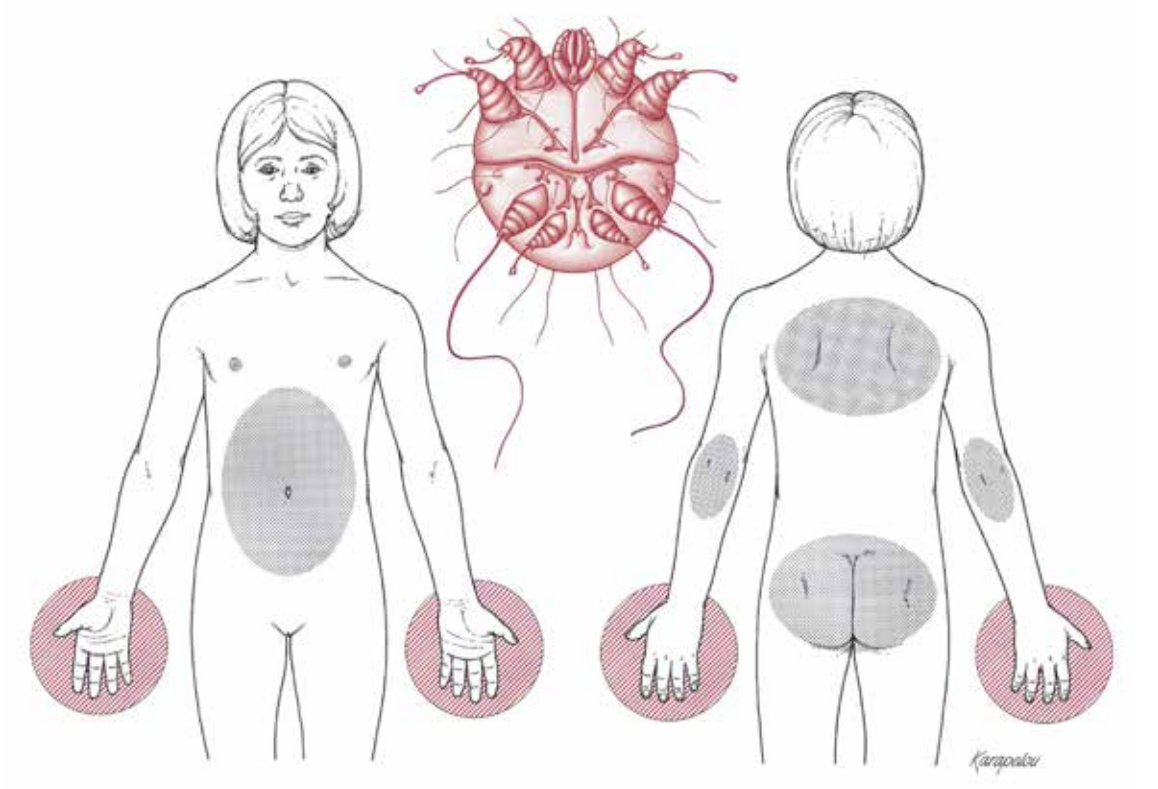

Figure 39.8. The itch mite, *Sarcoptes scabiei*. The stippled areas represent regions of the body where the rash is found. The mites themselves are found predominantly between the fingers and on the wrists (hatched areas).

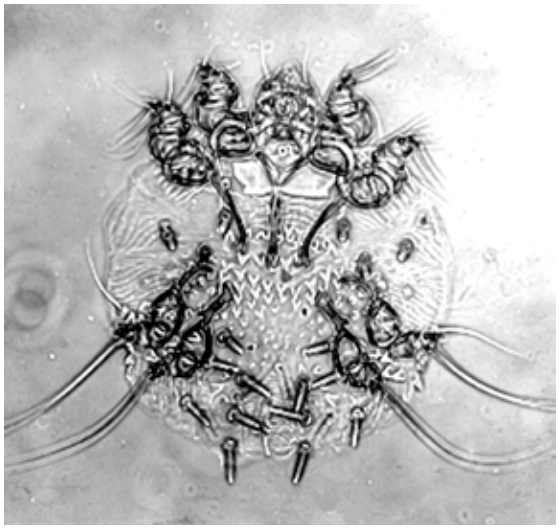

Figure 39.9. Adult female itch mite, *Sarcoptes scabiei.*

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 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]

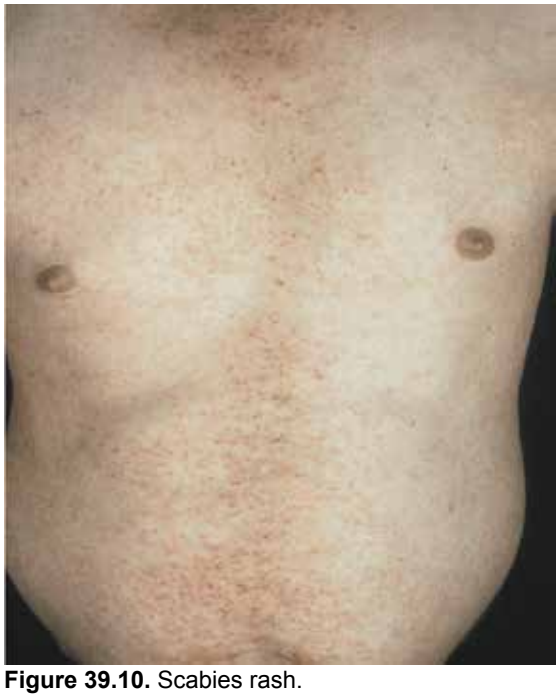

Figure 39.10. Scabies rash.

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. Mumcouglu.

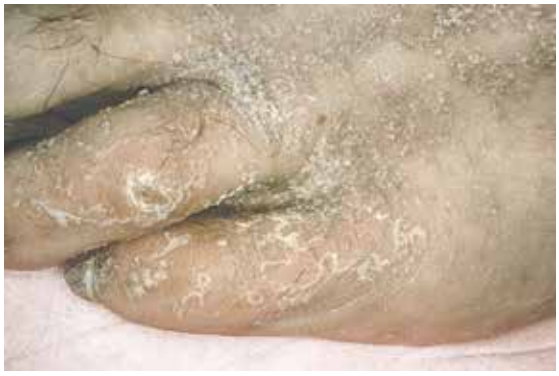

Figure 39.12. Large numbers of scabies mites and lesions on an immunosupressed patient.

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 poststreptococcal 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. *Loxoceles 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 *Loxoceles* in the New World attack when they are disturbed. Their bites may produce severe tissue reactions. *Loxoceles 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]

Loxoceles 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 *Loxoceles* 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 - *Loxoceles* 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 *Loxoceles* appears to work by inactivating hemolytic components of complement.[79-85] An antiserum for treatment of *Loxoceles* bites is being evaluated in Brazil. *Loxoceles* 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.

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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, *Blatella 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]

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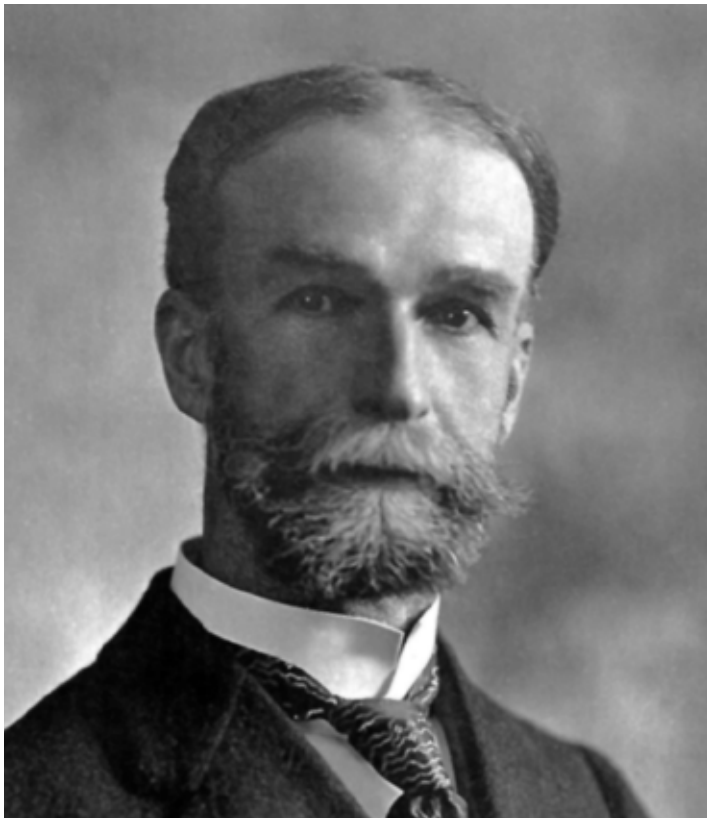


Theobald Smith, M.D. (1859–1934)

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 Stile'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)
Ethanol 95% or 100%	Two rinses
Ethanol 100%	1 minute
Xylol	1 minute

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.

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 H_2O.
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 H_2O.
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 H_2O.
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 H_2O.
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 H_2O.
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 H_2O.
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 H_2O in a 15-ml centrifuge tube.
2. Centrifuge for 1 minute at 2500 rpm; decant the supernatant.
3. Add zinc sulfate solution (specific gravity1.18) 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 H_2O.
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 H_2O at pH 7.4).
4. Destain 10–15 minutes in H_2O.
5. Air dry.
6. Examine microscopically.

Solutions

Schaudinn's Fixative

- $HgCl_2$, saturated aqueous solution: 666 ml (add 80 g H_gCl_2 to 1 liter de-ionized H_2O; 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 H_2O, 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)
- H_2O 900 ml

Adjust the pH of the solution to 7.0

Zinc Sulfate ($ZnSO_4$)

- $ZnSO_4$ 333 g
- H_2O (50–55˚C) 1000 ml

Adjust the specific gravity to 1.18 by adding either more H_2O or more zinc sulfate crystals.

Sugar Solution (Sheather's Method)

- Sucrose 500 g
- H_2O 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
- H_2O 250 ml

Store solution in a dark bottle.

Lugol's Iodine Solution

- Iodine 5 g
- Potassium iodide 10 g
- H_2O 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
- H_2O 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

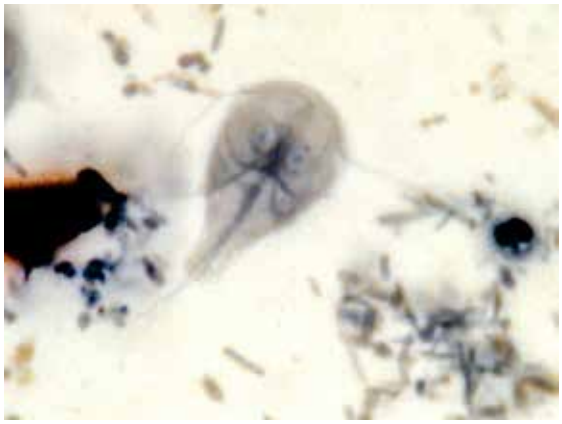


Figure C.1. *Giardia lamblia* trophozoite
15 µm

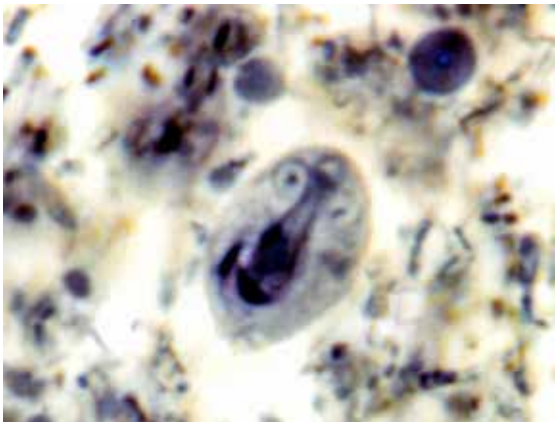


Figure C.2. *Giardia lamblia* cyst
15 µm

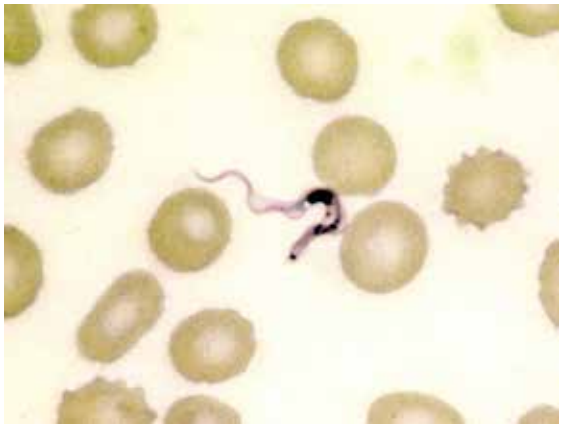


Figure C.3. *Trypanosoma brucei rhodesiense*
25 µm x 3 µm

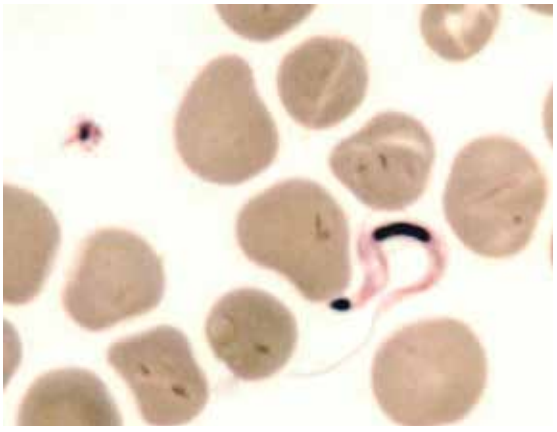


Figure C.4. *Trypanosoma cruzi*
20 µm x 3 µm

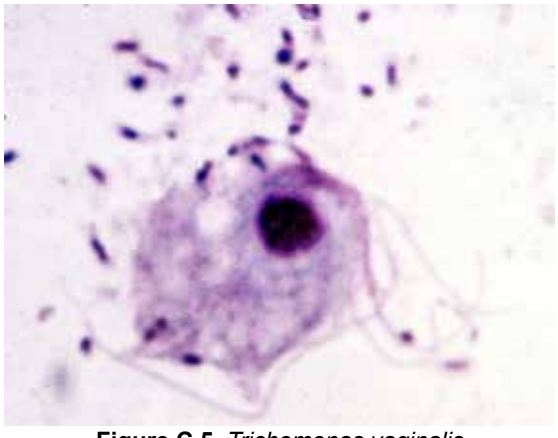


Figure C.5. *Trichomonas vaginalis*
20 µm x 10 µm

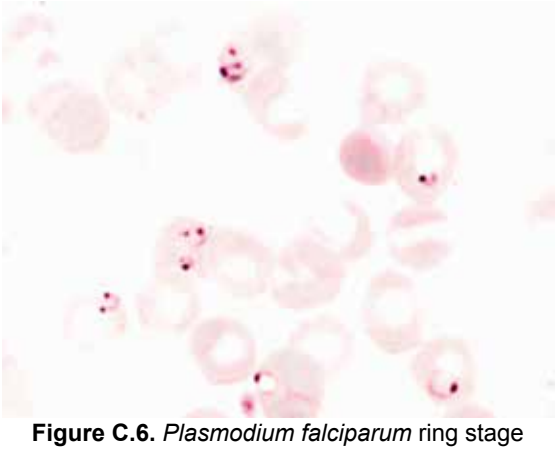


Figure C.6. *Plasmodium falciparum* ring stage

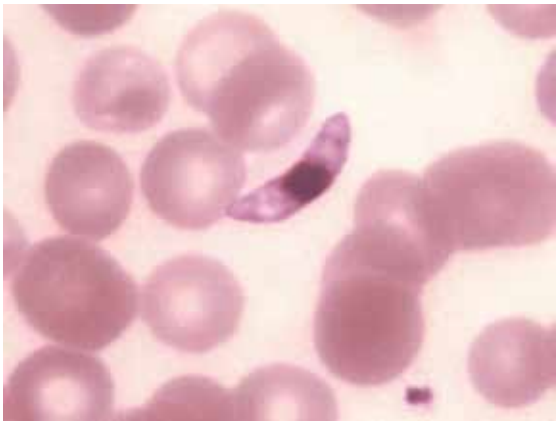


Figure C.7. *Plasmodium falciparum* macrogametocyte

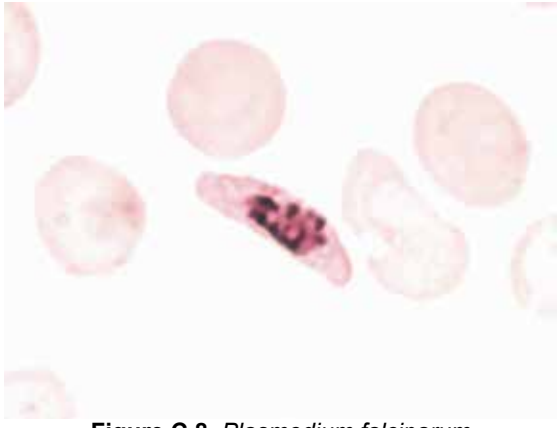


Figure C.8. *Plasmodium falciparum* microgametocyte

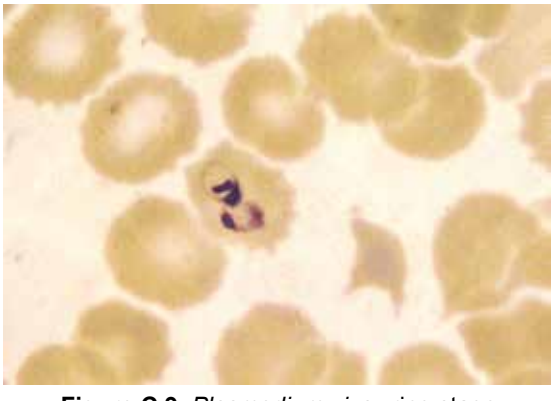


Figure C.9. *Plasmodium vivax* ring stage

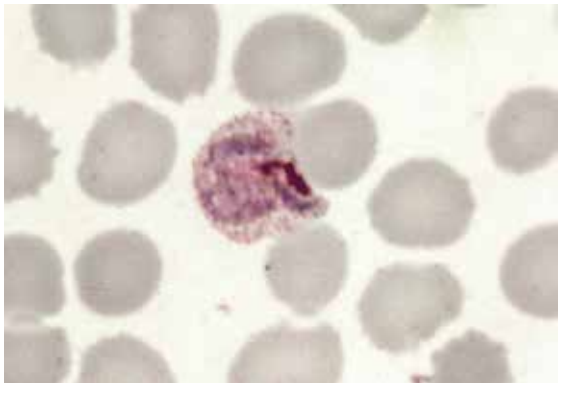


Figure C.10. *Plasmodium vivax* trophozoite

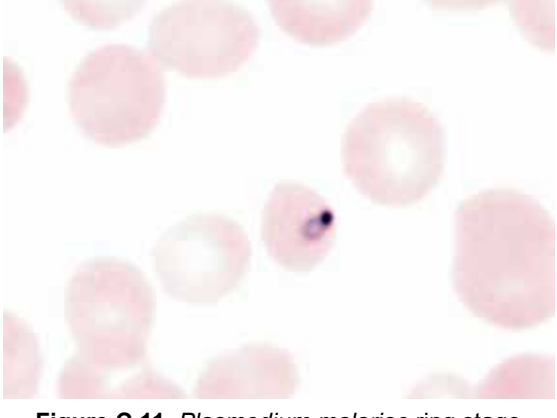


Figure C.11. *Plasmodium malariae* ring stage

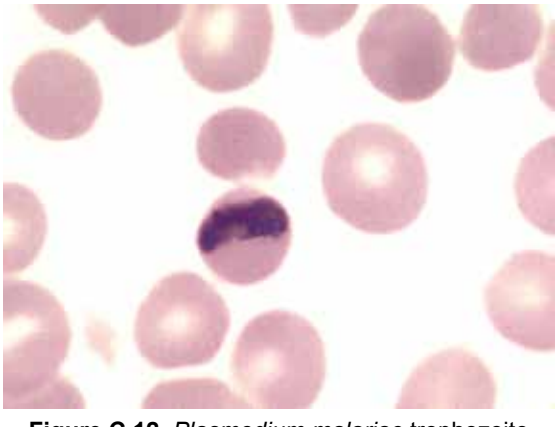


Figure C.12. *Plasmodium malariae* trophozoite

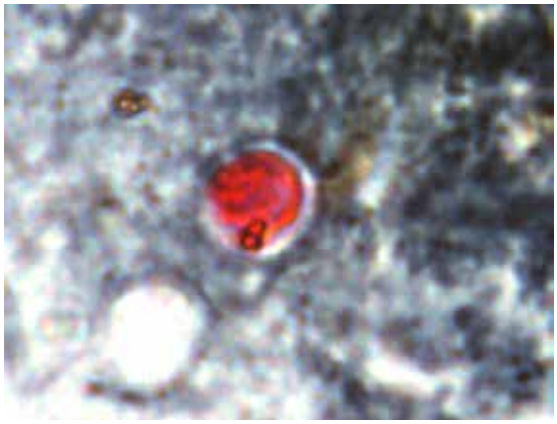


Figure C.13. *Cryptosporidium parvum* oocyst (acid-fast stain) 5 µm

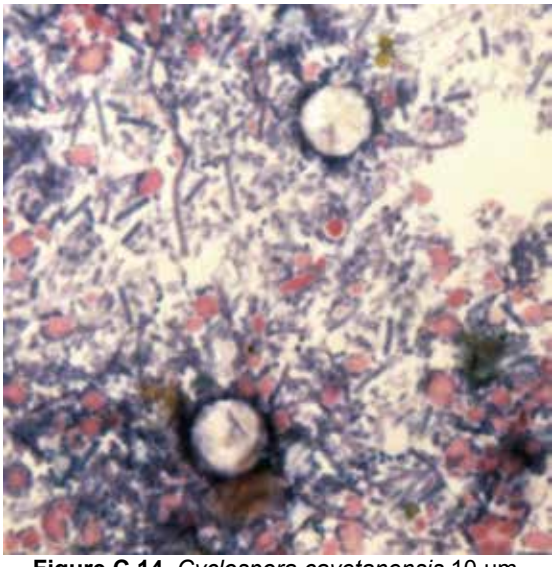


Figure C.14. *Cyclospora cayetanensis* 10 µm

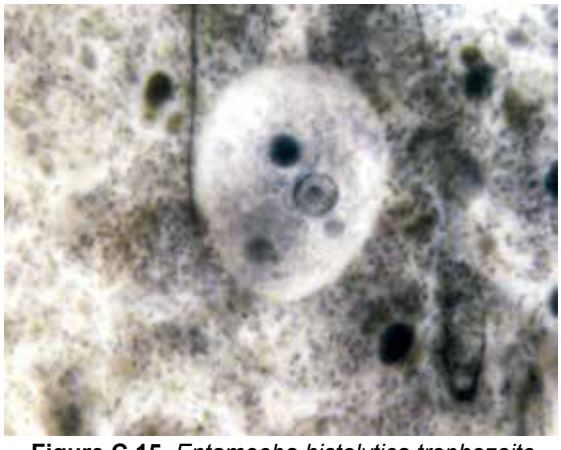


Figure C.15. *Entamoeba histolytica trophozoite* (note red cell in cytoplasm)

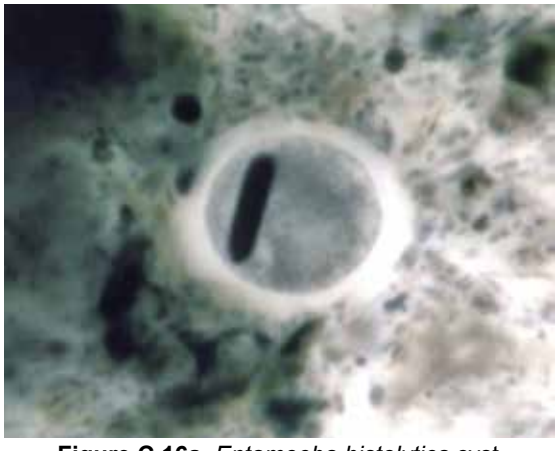


Figure C.16a. *Entamoeba histolytica* cyst Note smooth-ended chromatoidal bar 15 µm

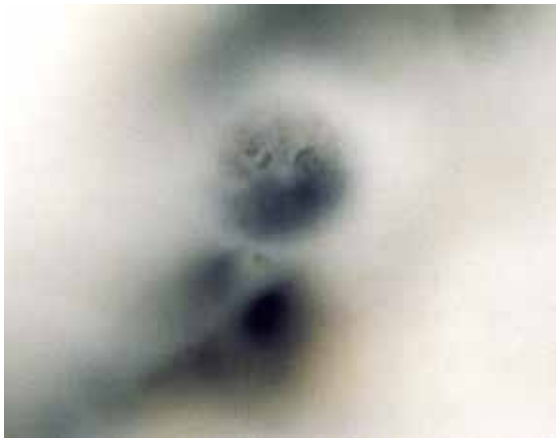


Figure C.16b. *Entamoeba histolytica* cyst (Through-focus #1): Note two nuclei 15 µm

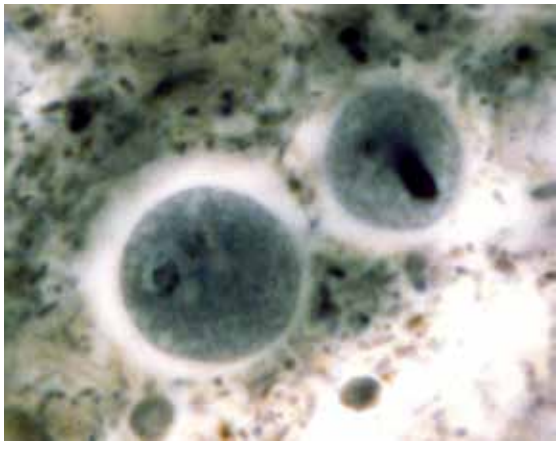


Figure C.16c. *Entamoeba histolytica* cyst (Through-focus #2): Note smooth-ended chromatoidal bar

Figure C.17. *Balantidium coli* trophozoite
150 µm (unstained)

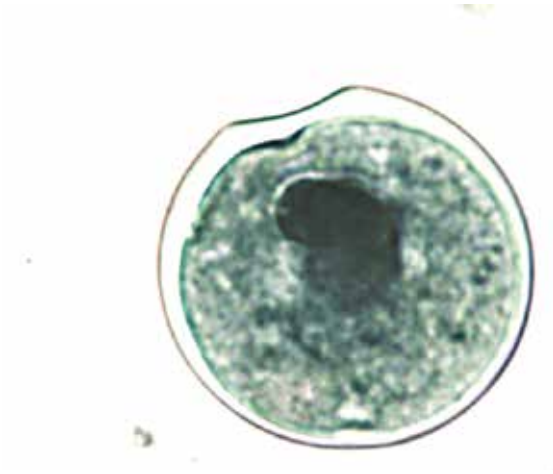


Figure C.18. *Balantidium coli* cyst
65 µm

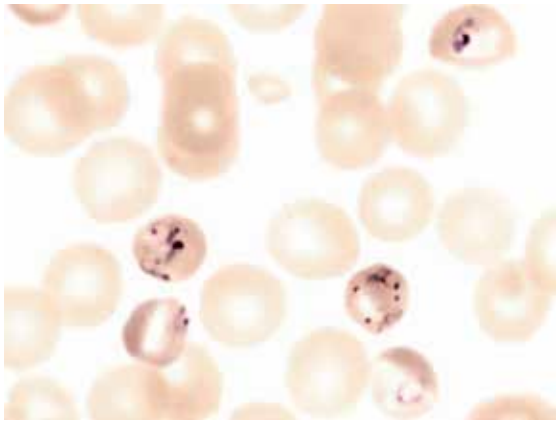


Figure C.19. *Babesia* spp. Bloodsmear

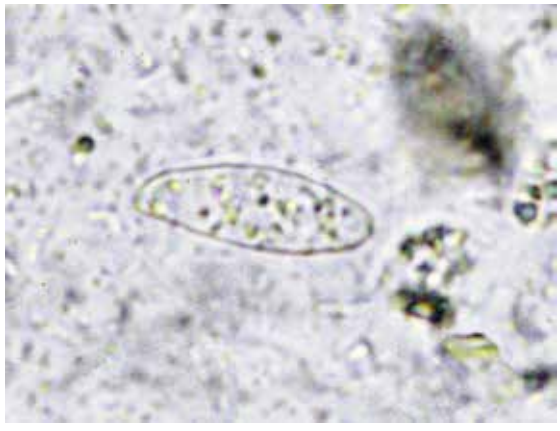


Figure C.20. *Cytoisospora belli* unsporulated oocyst
25 µm x 15 µm

Figure C.21. *Cytoisospora belli* sporulated oocyst
25 µm x 15 µm

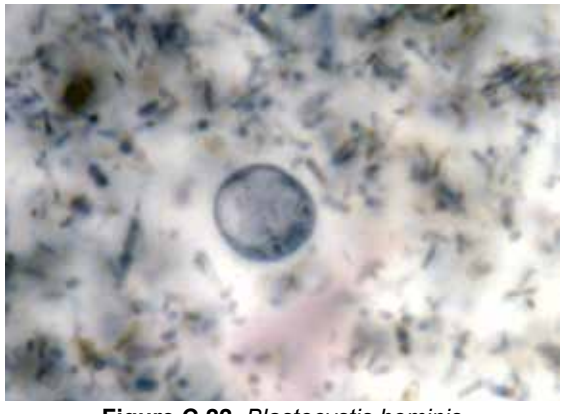


Figure C.22. *Blastocystis hominis*
6 µm

Figure C.23. *Trichomonas hominis*
10 µm x 8 µm

Figure C.24. *Trichomonas tenax*
7 µm x 3 µm

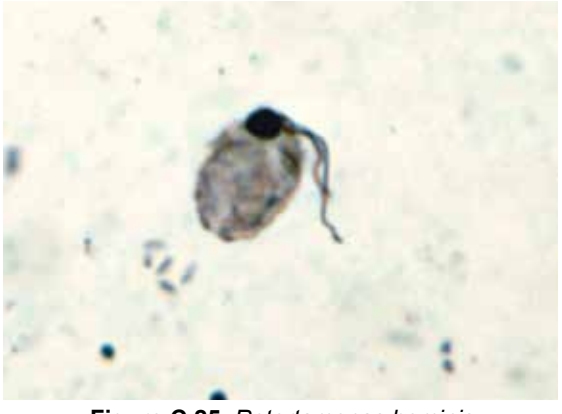


Figure C.25. *Retortamonas hominis*
6 µm x 2 µm

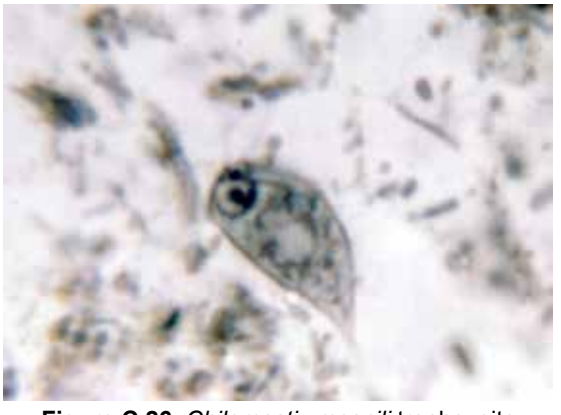


Figure C.26. *Chilomastix mesnili* trophozoite
15 µm x 12 µm

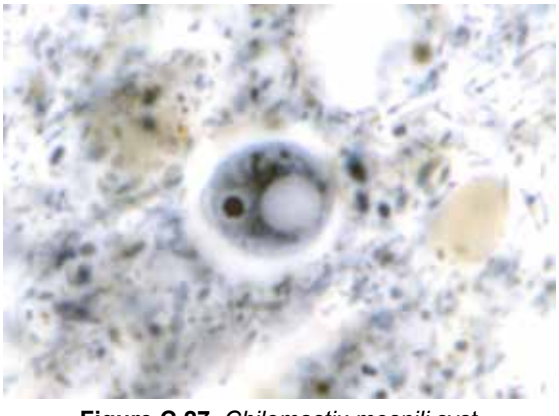


Figure C.27. *Chilomastix mesnili* cyst
8 µm x 5 µm

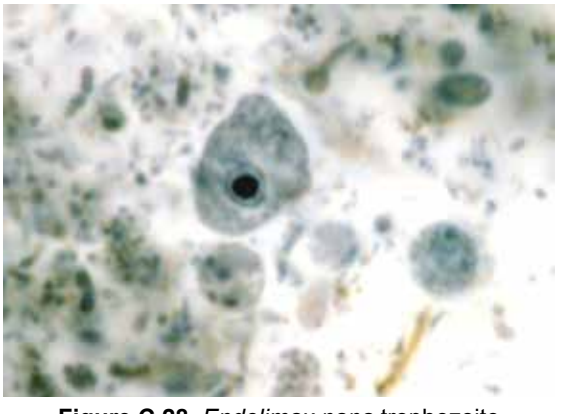


Figure C.28. *Endolimax nana* trophozoite
10 µm x 4 µm

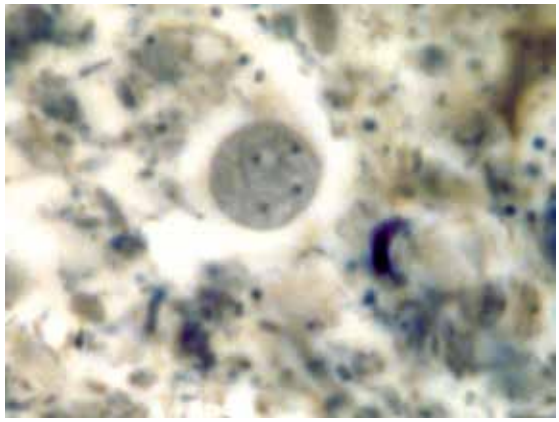


Figure C.29. *Endolimax nana* cyst
Note four nuclei
8 µm x 6 µm

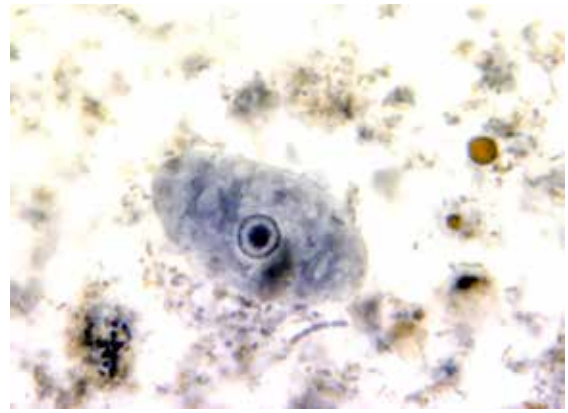


Figure C.30. *Iodamoeba bütschlii* trophozoite
18 µm

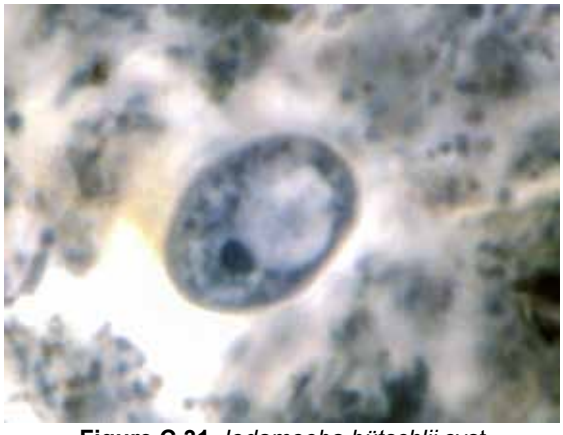


Figure C.31. *Iodamoeba bütschlii* cyst
12 µm x 8 µm

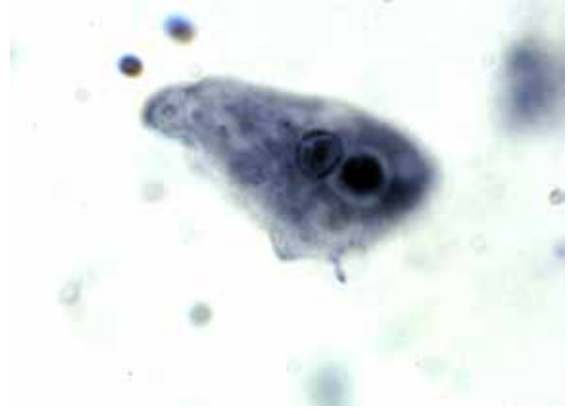


Figure C.32. *Entamoeba gingivalis* trophozoite
30 µm

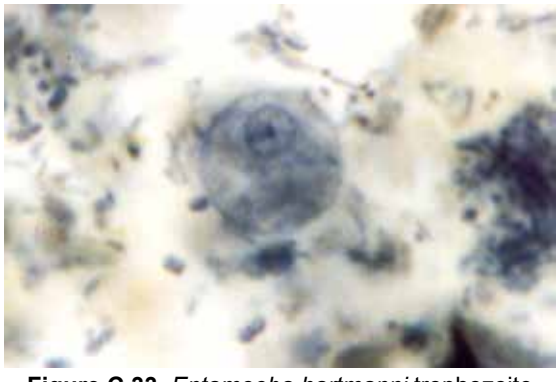


Figure C.33. *Entamoeba hartmanni* trophozoite
10 µm

Figure C.34. *Entamoeba coli* trophozoite
35 µm

Figure C.35a. *Entamoeba coli* cyst
(Through-focus #1): Two nuclei can be seen
30 µm

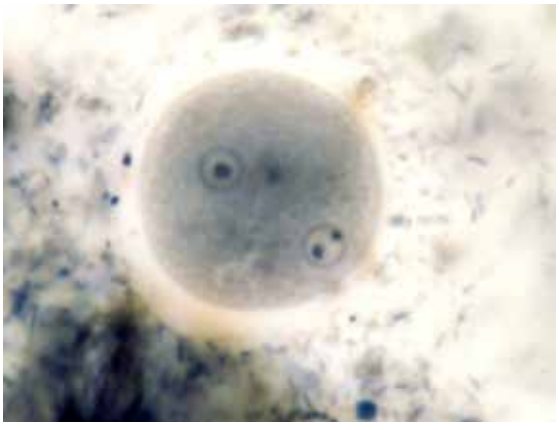


Figure C.35b. *Entamoeba coli* cyst
(Through-focus #2): Two nuclei can be seen
30 µm

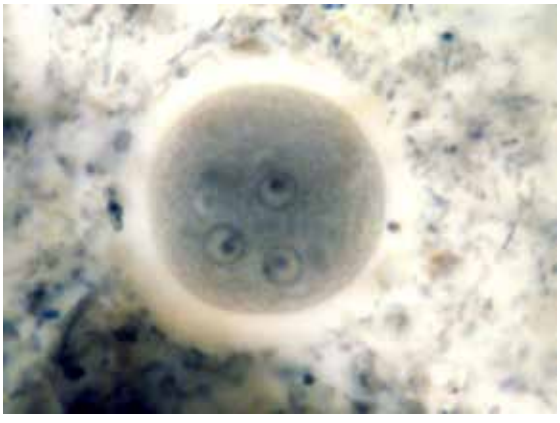


Figure C.35c. *Entamoeba coli* cyst
(Through-focus #3): Three nuclei can be seen
30 µm

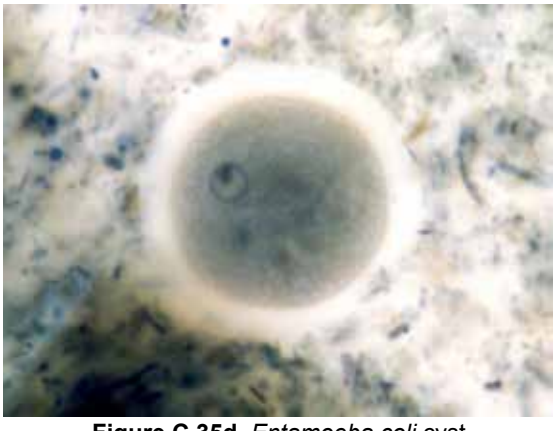


Figure C.35d. *Entamoeba coli* cyst
(Through-focus #4): One nucleus can be seen
A total of eight nuclei are present in the cyst
30 µm

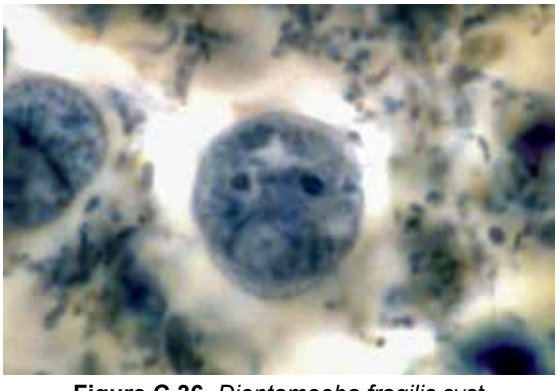


Figure C.36. *Dientamoeba fragilis* cyst
10 µm

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

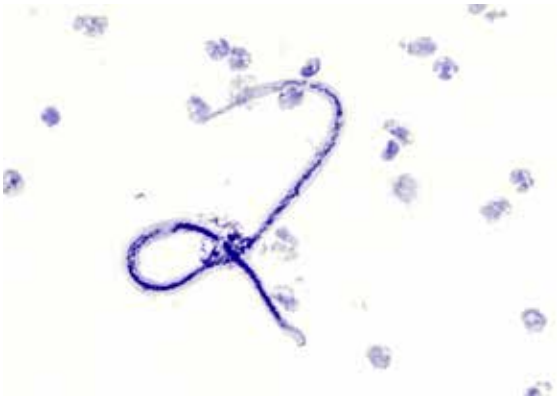


Figure C.46. *Wuchereria bancrofti* microfilaria
260 µm x 9 µm

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

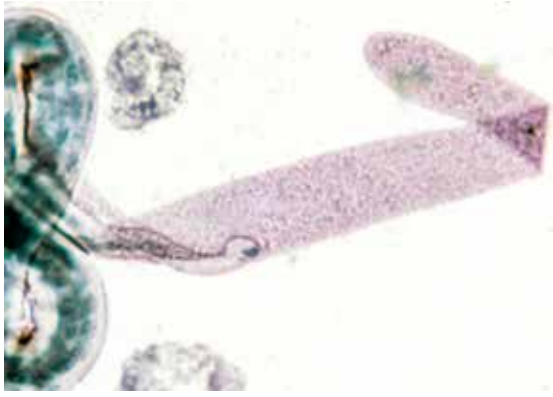


Figure C.49. *Brugia malayi* microfilaria
Note nucleus at tip of tail and sheath

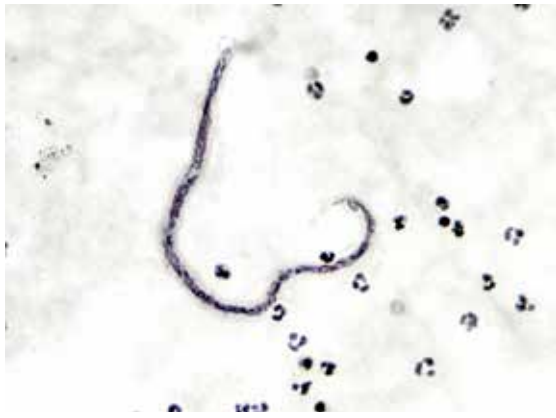


Figure C.50. *Loa loa* microfilaria
35 mm x 40 µm

Figure C.51. *Loa loa* microfilaria
Note sheath and nuclei, which extend to end of tail

Figure C.52. *Mansonella ozzardi* microfilaria
190 µm x 4 µm

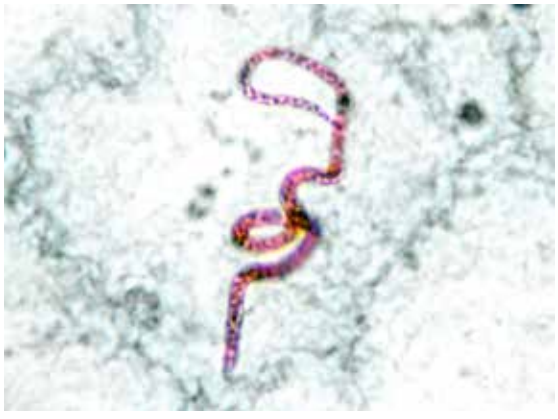


Figure C.53. *Mansonella perstans* microfilaria
200 µm x 4 µm

Figure C.54. *Capillaria philippinensis* ovum
40 µm x 20 µm

Figure C.55. *Capillaria hepatica* ovum
60 µm x 30 µm

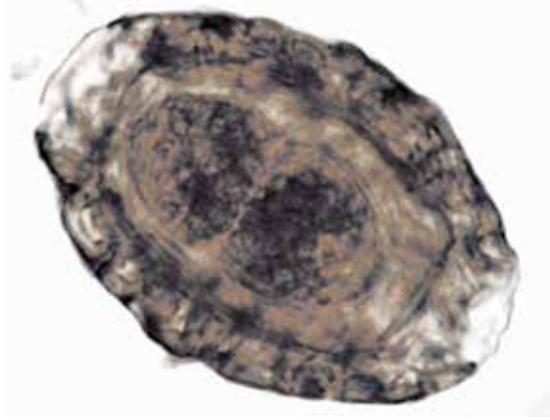


Figure C.56. *Dioctophyma renale* ovum
70 µm x 45 µm

Cestodes

Figure C.57. *Taenia* spp. ovum
40 µm x 30 µm

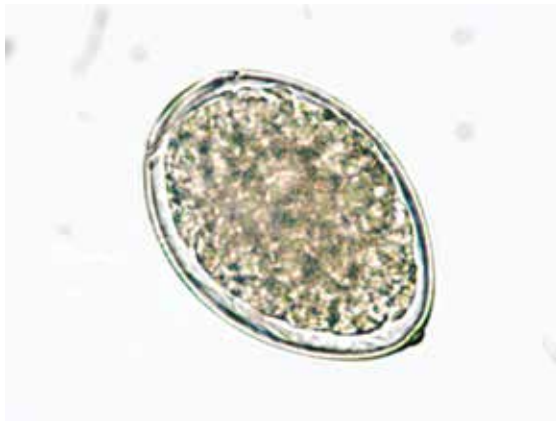


Figure C.58. *Diphyllobothrium latum* ovum
65 µm x 45 µm

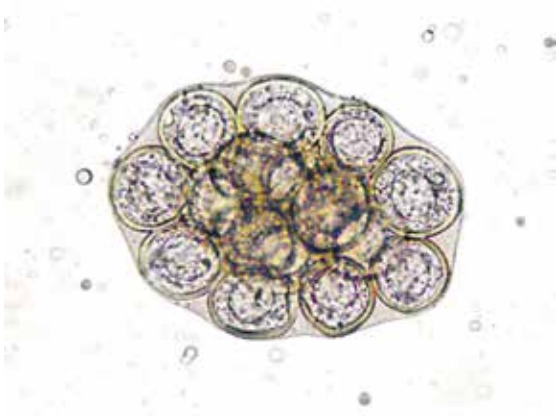


Figure C.59. *Dipylidum caninum* egg cluster
Each egg measures 35 µm

Figure C.60. *Hymenolepis nana* ovum
45 µm x 30 µm

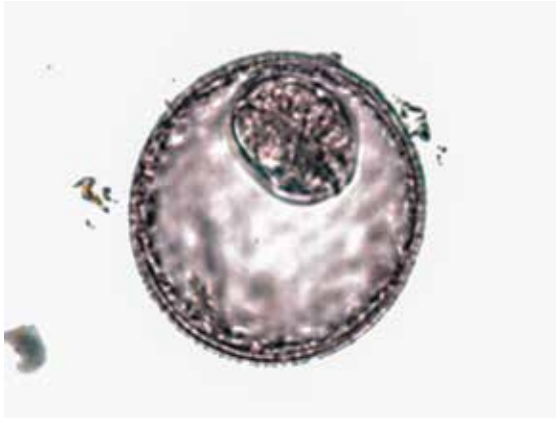


Figure C.61. *Hymenolepis diminuta* ovum
75 µm x 70 µm

Trematodes

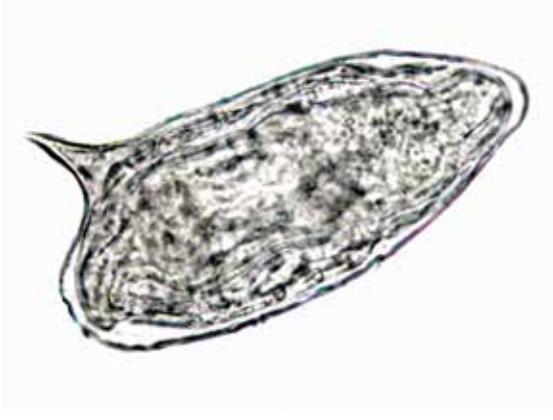


Figure C.62. *Schistosoma mansoni* ovum
160 µm x 60 µm

Figure C.63. *Schistosoma mansoni*
miracidium hatching from egg

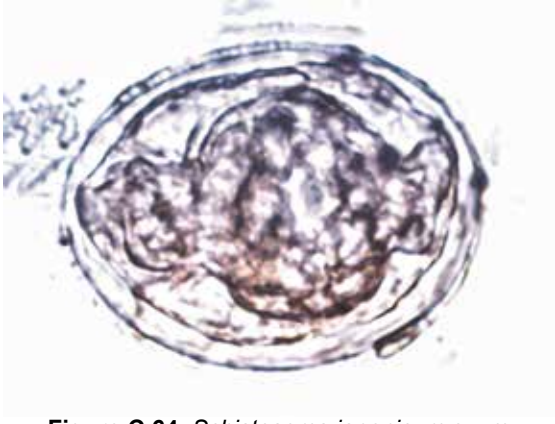


Figure C.64. *Schistosoma japonicum* ovum
85 µm x 60 µm

Figure C.65. *Schistosoma haematobium* ovum
170 µm x 60 µm

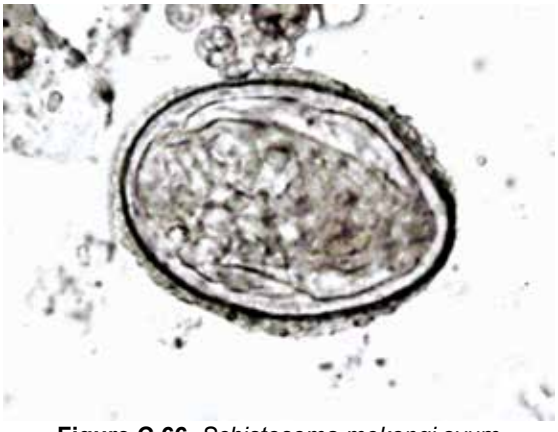


Figure C.66. *Schistosoma mekongi* ovum
60 µm x 45 µm

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

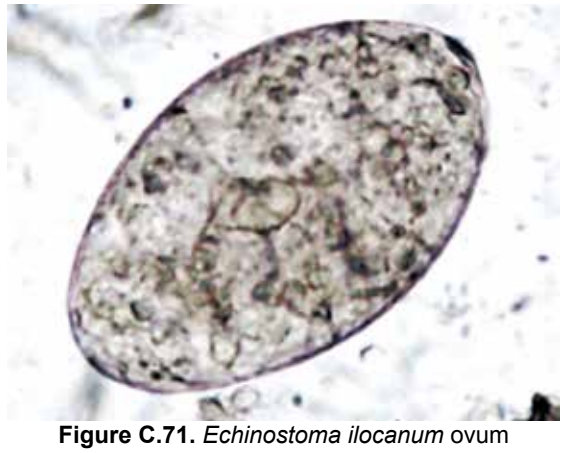


Figure C.71. *Echinostoma ilocanum* ovum
130 µm x 60 µm

Figure C.72. *Heterophyes heterophyes* ovum
20 µm x 15 µm

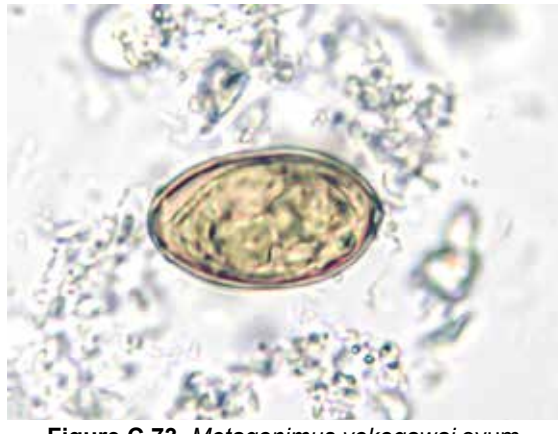


Figure C.73. *Metagonimus yokogawai* ovum
30 µm x 15 µm

Miscellaneous

Figure C.74. *Macrocanthorynchus hirudinaceus* ovum
60 µm x 20 µm

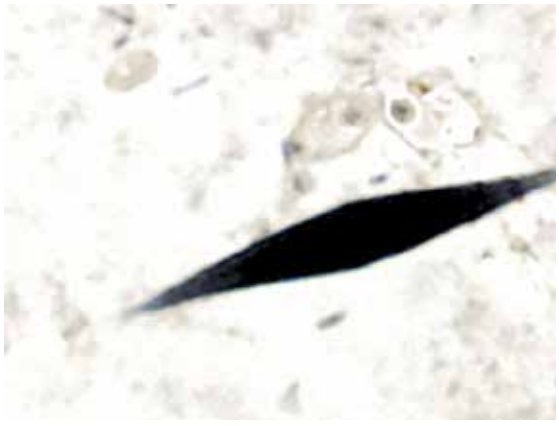


Figure C.75. Charcot-Leyden crystal
10 µm x 2 µm

Figure C.76. Digested vegetable matter
(Artifact)

Figure C.77. Plant fiber
(Artifact)

Figure C.78. Pollen grain
(Artifact)

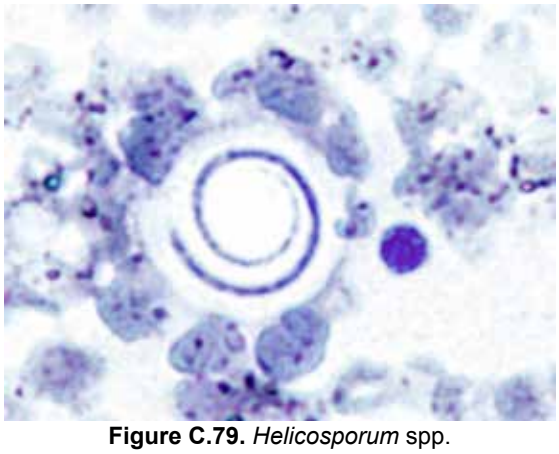
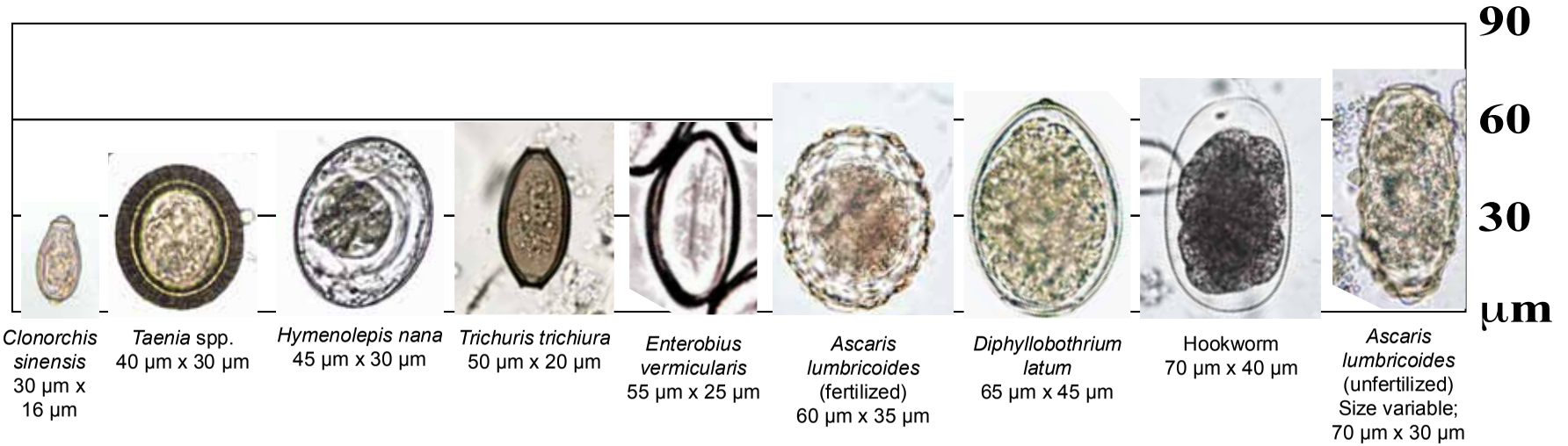


Figure C.79. *Helicosporum* spp.
(Fungus)

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.



Clonorchis sinensis
30 µm x 16 µm

Taenia spp.
40 µm x 30 µm

Hymenolepis nana
45 µm x 30 µm

Trichuris trichiura
50 µm x 20 µm

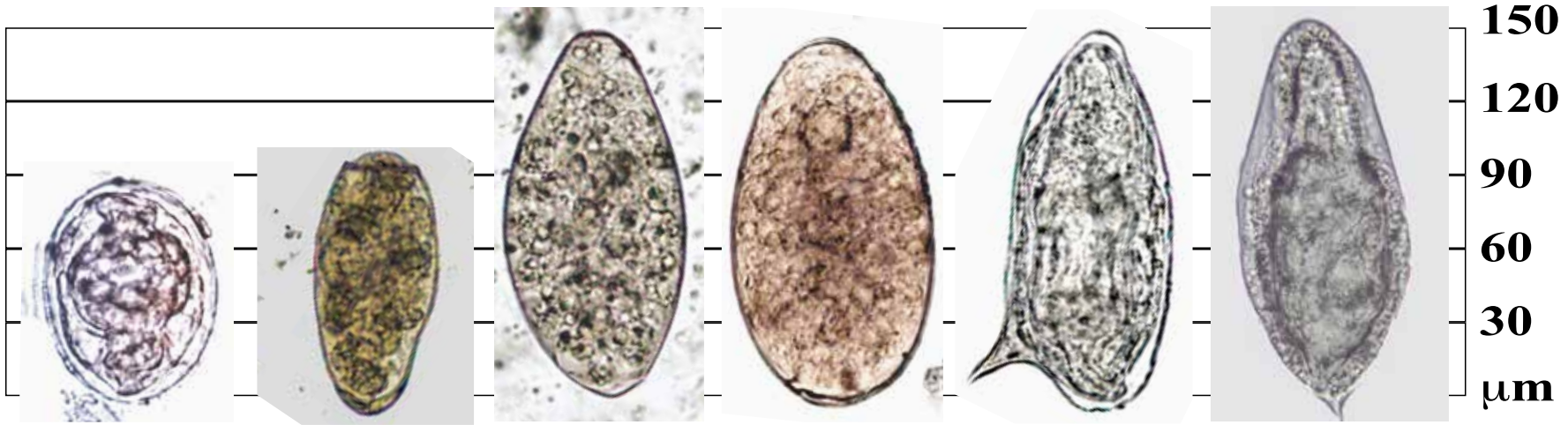
Enterobius vermicularis
55 µm x 25 µm

Ascaris lumbricoides
(fertilized)
60 µm x 35 µm

Diphyllobothrium latum
65 µm x 45 µm

Hookworm
70 µm x 40 µm

Ascaris lumbricoides
(unfertilized)
Size variable;
70 µm x 30 µm



Schistosoma japonicum
85 µm x 60 µm

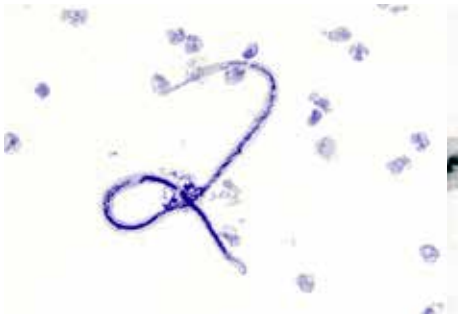
Paragonimus westermani
110 µm x 60 µm

Fasciolopsis buski
135 µm x 80 µm

Fasciola hepatica
140 µm x 75 µm

Schistosoma mansoni
160 µm x 60 µm

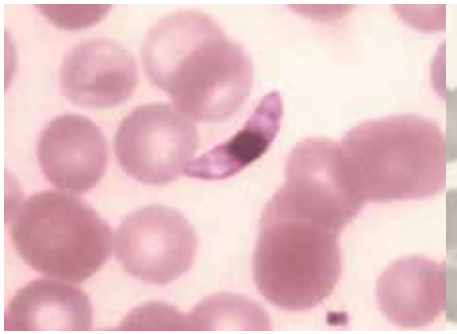
Schistosoma haematobium
170 µm x 60 µm



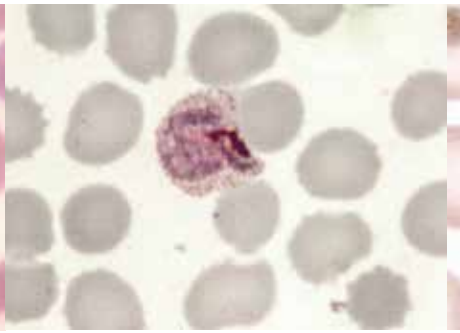
Wuchereria bancrofti microfilaria
260 µm x 9 µm

Wuchereria bancrofti microfilaria. Note sheath and nuclei, which do not extend to end of tail

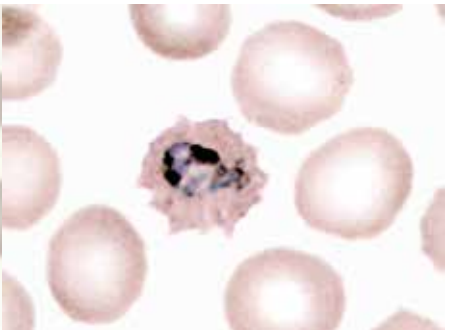
Loa loa microfilaria. Note sheath and nuclei, which extend to end of tail



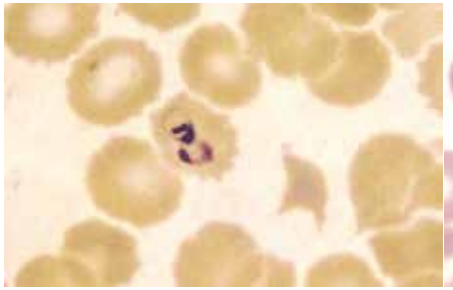
Gametocyte of *Plasmodium falciparum.*



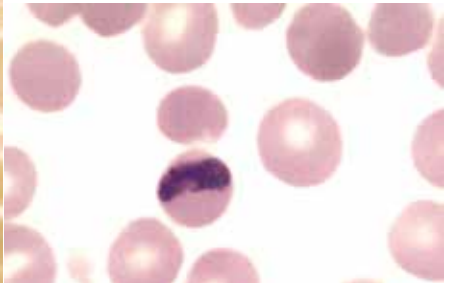
Trophozoite of *P. vivax*. Note Schüffner's dots in the parasite, and surrounding red cells that are smaller than the infected one.



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.

Clinical Appendix

1. *Giardia lamblia*

Exposure Required:
Oral ingestion of cysts in fecally contaminated water or food

Clinical Disease:
- Asymptomatic
- Acute diarrhea, chronic diarrhea, foul smelling diarrhea, floating stools, flatulence, nausea, rarely fever, epigastric pain, weight loss, fatigue

Diagnosis:
- Stool ova and parasites (microscopic examination of stool)
- Antigen capture-ELISA from stool sample
- NAAT

Treatment:
- **Preferred drug options:**
  - Metronidazole 250 mg PO TID x 7-days
  - Tinidazole 2 g PO x1
- **Alternate preferred drug option:**
  - Nitazoxanide 500 mg PO BID x 3 days
- **Alternative antimicrobials:**
  - Paromomycin 500 mg PO TID x 5–10 days
  - Furazolidone 100 mg PO QID x 7–10 days
  - Quinacrine 100 mg PO TID x 5–7 days
  - Albendazole 400 mg PO Q-day x 5–7 days

Resistant strains of *Giardia* are increasingly prevalent, but many will retreat with a different class of antimicrobial therapy or a longer course of the original agent. In some refractory cases combination antimicrobial therapy may be necessary.

3. Cutaneous Leishmaniasis

Exposure Required:
- Bite from sand fly

Clinical Disease:
- Small papule that then forms a painless chronic ulcer with a raised indurated border

Diagnosis:
Organisms are found only in the living tissue at the raised margin, regardless of the age of the lesion.
- Histology
- Culture
- NAAT
- Leishmaniasis skin test

Treatment:
For non-mucocutaneous species (see mucocutaneous section for those species with mucocutaneous potential).

- **Physical Therapies**
  - Thermotherapy – 50 °C once weekly for 4 weeks
  - CO_2 laser – single session
  - Photodynamic therapy – once weekly for 4 weeks after application of 'photosensitizer'
  - Cryotherapy – freeze for 10–30 s, thaw and perform 2–3 times, repeated every 1–4 weeks for 2–4 sessions or more depending on response
- **Local Drug Therapy**
  - Paromomycin ointment 15% -apply BID for 20–28 days
  - Clotrimazole 1% / miconazole 2% ointment applied BID x 30 days
  - Intralesional antimonials (meglumine antimoniate injected until complete blanching of border, every 3–7 days for 1–5 sessions-very painful!)
- **Oral Drug Therapy**
  - Azoles
    - Fluconazole 400 mg PO Q-day x 6 weeks
    - Ketoconazole 600 mg PO Q-day x 6 weeks
    - Itraconazole 400 mg PO Q-day x 3–6 weeks
  - Miltefosine (2.5 mg/kg/day) or for an adult 150 mg PO per day given as 50 mg 2–3x/day x 28 days
- **Parenteral Therapy**
  - Pentavalent antimonials
  - Amphotericin
  - Liposomal amphotericin
  - Pentamidine
- **Combination Therapy**
  - Combining local and parenteral
  - Combining different parenteral therapies

4. Mucocutaneous Leishmaniasis

Leishmania (V.) braziliensis ~ 30%
Leishmania (V.) panamensis ~ 10%
Leishmania (V.) guyanensis ~ 10%
Leishmania (L.) amazonensis (rare)

Exposure Required:
- Bite from sand fly

Clinical Disease:

- Small papule that then forms a painless chronic ulcer with a raised indurated border, followed by ulcers at mucous membranes – i.e. soft palate, nasal septum, larynx, anus, lips

Diagnosis:

Organisms are found only in the living tissue at the raised margin, regardless of the age of the lesion.

- NAAT – main modality and identifies species
- Histology – very low sensitivity
- Culture – very low sensitivity

Treatment:

For species with muco-cutaneous potential, even first infection.

- **Oral Drug Therapy**
  - Miltefosine (2.5 mg/kg/day) or for an adult 150 mg PO per day given as 50 mg 2–3x/day x 28 days
- **Parenteral Therapy**
  - Pentavalent antimonials
    - Sodium stibogluconate 20 mg/kg/day IV/IM x 28–30 days
    - Meglumine antimoniate 20 mg/kg/day IV/IM x 28–30 days
  - Amphotericin 0.5–1 mg/kg IV Q-day up to cumulative dose of 20–45 mg/kg
  - Liposomal amphotericin 3 mg/kg IV Q-day up to cumulative dose of 20–60 mg/kg
  - (Possible inferior option) pentamidine 2–4 mg/kg IV/IM every other day or 3x/week for 15 doses
- **Combination Therapy**
  - Combining local and parenteral
  - Combining different parenteral therapies

5. Visceral Leishmaniasis

Leishmania (L.) donovani
Leishmania (L.) infantum

Exposure Required:

- Bite from sand fly

Clinical Disease:

- Fever, lymphadenopathy, hepatomegaly, splenomegaly, weight loss, darkening of the skin

Diagnosis:

- NAAT – from marrow or splenic aspirate
- Culture
- Microscopic evaluation
- rK39 antigen ELISA

Treatment:

- **Parenteral Therapy**
  - Liposomal amphotericin 3–5 mg/kg IV Q-day for 3–5 days up to cumulative dose of 15 mg/kg or single 10 mg/kg IV dose, for HIV co-infected 3–5 mg/kg IV Q-day for 10 days up to cumulative dose of 40 mg/kg
  - Pentavalent antimonials (preferred in Africa but not in India)
    - Sodium stibogluconate 20 mg/kg/day IV/IM x 28–30 days
    - Meglumine antimoniate 20 mg/kg/day IV/IM x 28–30 days
  - Amphotericin 0.5–1 mg/kg IV Q-day up to cumulative dose of 20–45 mg/kg
  - Liposomal amphotericin 3 mg/kg IV Q-day up to cumulative dose of 20–60 mg/kg

- **Oral Drug Therapy**
  - (Non-preferred-alternative) miltefosine (2.5 mg/kg/day) or for an adult 150 mg PO per day given as 50 mg 2–3x/day x 28 days

- **Combination Therapy**
  - Combining amphotericin and miltefosine

6. African Trypanosomiasis

Trypanosoma brucei rhodesiense
Trypanosoma brucei gambiense

Exposure Required:

- Bite from tsetse fly

Clinical Disease:

- Hemolymphatic stage – initially a large painless chancre at bite site, rash, generalized pruritus, weight loss, facial swelling, posterior cervical adenopathy
- CNS Stage – headache, stiff neck, periods of insomnia alternating with hypersomnolence, depression, seizures, tremors, palsies, coma

Diagnosis:

- Microscopic
  - Blood smears
  - Lymph node aspirates
  - CSF - examination of CSF is mandatory in the diagnostic evaluation of trypanosomiasis and a white blood cell count >5 cells/ml is considered indicative of CNS involvement
  - Aspirates taken at the edge of chancres
  - NAAT –limited availability
- Cultures
- Card agglutination test for trypanosomiasis

Treatment:
Treatment is determined by species and stage.

- **Early Stages:**
  Trypanosoma b. gambiense
  - (Preferred) pentamidine 4 mg/kg/day IV/IM (given over 2 hrs) for 7 days
  - (Alternative) suramin 100–200 mg test dose, then 20 mg/kg (max 1g) IV on days 1, 3, 7, 14, and 21

  Trypanosoma b. rhodesiense
  - Suramin 100–200 mg test dose, then 20 mg/kg (max 1g) IV on days 1, 3, 7, 14, and 21
- **Late Stages:**
  Trypanosoma b. gambiense
  - (Preferred) eflornithine 200 mg/kg IV q12 hrs (given over 1 hr) for 7 days PLUS nifurtimox 5 mg/kg PO q8 hrs for 10 days
  - Eflornithine 100 mg/kg IV q6 hrs for 14 days (monotherapy)
  - (Alternative) melarsoprol 2.2 mg/kg/day IV x 10 days (with oral prednisone)

  Trypanosoma b. rhodesiense
  - (Preferred) melarsoprol 2.2 mg/kg/day IV q10 days (with oral prednisone)
  - (Alternative) melarsoprol; three series of 3.6 mg/kg/day IV x 3 days spaced apart by 7-day intervals (with oral prednisone)

7. American Trypanosomiasis

Trypanosoma cruzi

Exposure Required:

- Trypomastigotes present in reduviid bug feces enter through bug bite or mucous membranes, ingestion orally (contaminated sweetened drinks), transfusion, congenital

Clinical Disease:

- Acute – malaise, fever, myocarditis, pericardial effusion, meningoencephalitis
- Chronic – cardiac damage (arrhythmias, congestive heart failure), gastrointestinal (GI) damage (dysphagia, regurgitation, megacolon, constipation)

Diagnosis:

- Microscopic - blood smears
- NAAT - limited availability
- Serology
- Histology

Treatment:

- Benznidazole (for 60 days) through the Centers for Disease Control (CDC) and Prevention
  - Adults (>12 years old) 5–7.5 mg/kg daily divided into BID doses (12.5 and 100 mg tablets)
  - Children (< 12 years old) 5 mg/kg PO BID

- Nifurtimox (for 90 days) (divided into 3–4 doses per day)
  - Adults 8–10 mg/kg/day
  - Young children (<10 years old) 15–20 mg/kg/day
  - Older children (>10 years old <16 years old)12.5–15 mg/kg/day

8. *Trichomonas vaginalis*

Exposure Required:
- Acquisition of trophozoites during sexual contact or at birth

Clinical Disease:
- Asymptomatic
- Discomfort, dyspareunia, vaginal discharge (thick, yellow, blood tinged, pH increased from 4.5 to >5.0), strawberry cervix, erythema, dysuria

Diagnosis:
- Microscopic observation of motile forms from wet prep
- Culture
- Rapid antigen testing
- Nucleic acid probe test
- NAAT

Treatment:
- Metronidazole 500 mg PO BID x 7 days
- Tinidazole 2 g PO x1
- Combination: paromomycin compounded cream 250 mg each day with high dose tinidazole 1 g orally 3x/day for 2 weeks

9. The Malarias

Plasmodium falciparum
Plasmodium vivax
Plasmodium ovale
Plasmodium malariae
Plasmodium knowlesi

Exposure Required:
- Bite from female *Anopheles* mosquito

Clinical Disease:
- Periodic fever and chills, cough, abdominal pain, vomiting, diarrhea, dyspnea, anemia, leukopenia, eosinopenia, thrombocytopenia,

Diagnosis:
- Thick and thin blood smears
- Antibody-based rapid diagnostic tests
- NAAT
- Mutation-specific PCR

Staging:

- Uncomplicated malaria (not having a feature of severe)
- Severe malaria (having one or more of the following features)
    - Decreased level of consciousness
    - Unable to sit or stand without assistance
    - Convulsions (more than 2 in <24 hrs)
    - Acidosis or bicarbonate <15 mmol/L
    - Hypoglycemia (specific cutoffs)
    - Anemia (specific cutoffs)
    - Renal impairment (Cr >3 mg/dL or BUN >20 mmol/L
    - Jaundice (bilirubin >3 mg/dL)
    - Pulmonary edema (observable with chest X-ray, hypoxemia, tachypnea)
    - Significant bleeding
    - Shock
    - *P. falciparum* parasitemia >10%

Treatment:

- **Chloroquine-Sensitive Uncomplicated Malaria (not having a feature of severe)**
    - Chloroquine
        - Adults 1 g PO x1 then 500 mg at 6, 24, and 48 hrs
        - Children 10 mg/kg followed by 5 mg/kg of the base at 6, 24, and 48 hrs
    - Hydroxychloroquine
        - Adults 800 mg x1, then 400 mg at 6, 24, and 48 hrs
        - Children 10 mg/kg x1, then 5 mg/kg at 6, 24, and 48 hrs

- **Chloroquine-resistant Uncomplicated Malaria (not having a feature of severe)**
    - Artemisinin combination therapy (see table below)
    - Atovaquone-proguanil (adults) 4-tablets PO Q-Day x 3 days
    - Quinine 650 mg PO TID for 3–7 days plus second agent for 7 days PLUS (doxycycline, or tetracycline, or clindamycin)
    - Mefloquine 3-tablets PO x1, then 2 tablets 6 hrs later

- **Treatment of Complicated Malaria**
    - Artemisinin derivatives
        - Artesunate IV (preferred) (If>20 kg) 2.4 mg/kg IV x1 then at 12 hrs, 24 hrs, then daily, (If <20 kg) 3 mg/kg IV same schedule
        - Artemether IM 3.2 mg/kg IM x1 then 1.6 mg/kg Q-day
    - Quinidine based
        - Quinidine gluconate 10 mg/kg IV x1 then continuous infusion 0.0125 mg/kg/min
        - Plus, doxycycline or clindamycin for 7 days

- **Treatment to Prevent Malaria Relapse (Hyponozoites)**
    - Primaquine – 30 mg (of base) 2-tablets PO Q-day for 2 weeks

Consider broad spectrum antibiotics (~10% coinfected)/Exchange transfusion not recommended

Medication	Forms available	Weight (kg)-Dose(mg)
Artemether-lumefantrine	20/120mg 40/240mg	<15kg 20/120, 15-25kg 40/240, 25-35kg 60/360, >35kg 80/480 (PO BID x 3 days)
Artesunate-amodiaquine	25/67.5mg 50/135mg 100/270mg and separate tablets	<9kg 25/67.5, 9-18kg 50/135, 18-36kg 100/270, >36kg 200/540 (PO Q-day x 3 days)
Artesunate-mefloquine	25/55mg 100/220mg and separate tablets	<9kg 25/55, 9-18kg 50/110, 18-30kg 100/220, >30kg 200/440 (PO Q-day x 3 days)
Artesunate-sulfadoxine-pyrimethamine	Artesunate(A) 50mg and SP 500/25mg	<10kg SP-250/12.5 PO x1 day#1+A-25 PO Q-day x 3days, 10-25kg SP-500/25 PO x1 day#1+A-50 PO Q-day x 3days, 25-50kg SP-1000/50 PO x1 day#1+A-100 PO Q-day x 3days, >50kg SP-1500/75 PO x1 day#1+A-200 PO Q-day x 3days
Dihydroartemisinin-piperaquine	20/160mg 40/320mg and separate tablets	<8kg 20/160, 8-11kg 30/240, 11-17kg 40/320 17-25kg 60/480, 25-36kg 80/640, 36-60 120/960, 60-80kg 160/1280, >80kg 200/1600 (PO Q-day x 3days)

10. *Cryptosporidium parvum*

Exposure Required:

- Oral ingestion of oocysts in fecally contaminated water or food, rarely aerosol

Clinical Disease:

- Watery diarrhea, upper abdominal cramps, anorexia, nausea, weight loss and vomiting

Diagnosis:

- Stool microscopy
- PCR testing
- Antigen tests
- Multiplex NAAT testing

Treatment:

Limited evidence for benefit from any specific therapy so main focus is on restoration of immune dysfunction, if present, and rehydration.

- Nitazoxanide 500 mg PO TID for 3–14 days

11. *Toxoplasma gondii*

Exposure Required:

- Oral ingestion of pseudocysts in undercooked or raw meat, or oocysts from cat feces, and vertical transmission from mother to child during pregnancy

Clinical Disease:

- Congenital – chorioretinitis, hydrocephalus, intracranial calcifications, hepatomegaly, liver failure, thrombocytopenia, seizures, cognitive deficiencies
- Acute – mono-like illness, fever, cervical adenopathy, fatigue
- Reactivation in immunocompromised patient – encephalitis, ring enhancing cerebral lesions, interstitial pneumonitis

Diagnosis:
- Microscopy
- NAAT
- Specific immunoglobulins
- Imaging

Treatment:
- (If < 60kg) pyrimethamine 100–200 mg x1 then 50 mg PO daily + sulfadiazine 1,000 mg PO QID + leukovorin 10–25 mg PO daily
- (If > 60 kg) pyrimethamine 100–200 mg x1 then 75 mg PO daily + sulfadiazine 1,500 mg PO QID + leukovorin 10–25 mg PO daily
- (If pyrimethamine is unavailable then TMP-SMX 5 mg/kg based on TMP component IV/PO BID)
- Pregnancy- unclear on best approach but spiramycin has been used during the first trimester

12. *Entamoeba histolytica*

Exposure Required:
- Oral ingestion of cysts in fecally contaminated water or food

Clinical Disease:
- Intestinal – acute bloody diarrhea (dysentery but blood may only be microscopic), less commonly chronic diarrhea, abdominal discomfort, amoeboma (colonic mass)
- Extra-intestinal – liver lesions, pulmonary lesion, less commonly (pleura, cardiac and cerebral)

Diagnosis:
- Antigens
- NAAT
- Microscopy coupled with species identification by one of the first two testing modalities

Treatment:
- **Intestinal**
  - (Preferred) metronidazole 500 mg PO TID for 10 days
  - (Alternative) tinidazole 2 g PO Q-day x 3 days
  - (Alternative) nitazoxanide 500 mg PO BID x 3 days
  - (Luminal agents) iodoquinol 650 mg PO TID x 20 days or, paromomycin 1,000 mg PO TID x 7 days or diloxanide 500 mg PO TID x 10 days
- **Extra-intestinal**
  - (Preferred) metronidazole 500 mg PO TID for 10 days
  - (Alternative) tinidazole 2 g PO Q-day x 5 days
  - (Alternative) nitazoxanide 500 mg PO BID x 10 days
  - (Luminal agents) iodoquinol 650 mg PO TID x 20 days or, paromomycin 1,000 mg PO TID x 7 days or diloxanide 500 mg PO TID x 10 days

13. *Balantidium coli*

Exposure Required:
- Oral ingestion of cysts in fecally contaminated water or food

Clinical Disease:
- Asymptomatic, watery diarrhea, dysentery, fever, nausea, vomiting, malaise

Diagnosis:
- Microscopy

Treatment:
- Tetracycline 500 mg PO 4x/day x 10 days
- Metronidazole 750 mg PO TID x 5 days
- Iodoquinol, paromomycin, nitazoxanide and chloroquine have been used

14. Other Protozoa of Medical Importance

A. *Babesia spp.*

Exposure Required:
- Bite of larval *Ixodes scapularis* tick (black legged deer tick)

Clinical Disease:
- Fever, malaise, headache, bradycardia, lymphopenia, anemia

Diagnosis:
- Microscopy (blood smears) rarely a 'Maltese cross' can be seen
- NAAT
- Serology

Treatment:
- **Immunocompetent**
  - Mild to Moderate Disease:
    - (Preferred) azithromycin 500 mg PO day 1 then 250 mg PO Q-day PLUS atovaquone 750 mg PO BID for 7–10 days
    - (Alternative) clindamycin 600 mg PO TID PLUS quinine 650 mg PO TID for 7–10 days
  - Severe Disease (consider exchange transfusion):
    - (Preferred) azithromycin 500 mg IV Q-day PLUS atovaquone 750 mg PO BID for 7–10 days
    - (Alternative) clindamycin 300–600 mg IV TID PLUS quinine 650 mg PO TID for 7–10 days

- **Immunocompromised**
  - Mild to Moderate Disease:
    - (Preferred) azithromycin 1000 mg PO Q-day PLUS atovaquone 750 mg PO BID for 7–10 days or longer
    - (Alternative) clindamycin 600 mg PO TID PLUS quinine 650 mg PO TID for 7–10 days or longer

- Severe Disease (consider exchange transfusion):
  - (Preferred) azithromycin 500 mg IV Q-day PLUS atovaquone 750 mg PO BID for 7–10 days or longer
  - (Alternative) clindamycin 300–600 mg IV TID PLUS quinine 650 mg PO TID for 7–10 days or longer

B. *Cystoisospora belli*

Exposure Required:
- Oral ingestion of oocysts in fecally contaminated water or food (not direct person to person as 1–2 days required for sporulation)

Clinical Disease:
- Fever, abdominal cramping, watery non-bloody diarrhea, malaise, weight loss, eosinophilia

Diagnosis:
- Stool microscopy –requires acid fast staining or specific fluorescent techniques
- NAAT

Treatment:
- **Immunocompetent**
  - Trimethoprim-sulfamethoxazole (TMP-SMX) double strength 160/800 mg PO BID for 7–10 days
- **Immunocompromised**
  - (Preferred) trimethoprim-sulfamethoxazole (TMP-SMX) double strength 160/800 mg PO BID for 14 days followed by 1-tab PO 3 times per week
  - (Alternative-inferior) pyrimethamine with leucovorin or nitazoxanide may have activity
  - (Alternative-inferior) ciprofloxacin 500 mg PO BID for 14 days

C. *Cyclospora cayetanensis*

Exposure Required:
- Oral ingestion of oocysts in fecally contaminated water or food

Clinical Disease:
- Watery diarrhea

Diagnosis:
- Microscopy (improved sensitivity with acid-fast staining)
- NAAT

Treatment:
- (Preferred) trimethoprim-sulfamethoxazole (TMP-SMX) double strength 160/800 mg PO BID for 7–10 days
- (Alternative) nitazoxanide 500 mg PO BID for 7 days
- (Alternative-inferior) ciprofloxacin 500 mg PO BID x 7 days

D. *Naeglaria fowleri*

Exposure Required:
- Contact of the inside of the nasal cavity (cribriform plate) with water containing trophozoites

Clinical Disease:
- Frontal headache, vomiting, confusion, fever, coma

Diagnosis:
- Microscopy/histology
- NAAT

Treatment: (multidrug regimen)
- Conventional-amphotericin (not liposomal) 1.5 mg/kg/day IV, and
- Rifampin 10 mg/kg/day PO in three doses, and
- Fluconazole 10 mg/kg/day IV or PO, and
- Miltefosine 50 mg PO BID or TID, and
- Azithromycin 500 mg Q-day IV or PO

E. *Acanthamoeba* spp.

Exposure Required:
- Most likely acquired through lungs or skin on exposure to keratitis contaminated tap water

Clinical Disease:
- Encephalitic form- Granulomatous Amebic Encephalitis (GAE) - frontal headache, diplopia, seizures
- Ulcerative keratitis – gritty feeling in eye, impaired vision, blindness

Diagnosis:
- Microscopy identification of trophozoites on wet mount, by confocal, or in fixed specimens
- Culture
- NAAT

Treatment:
- Keratitis – often combination therapy
- Disseminated disease (skin, CNS, disseminated) – use combination therapy

F. *Balamuthia mandrillaris*

Exposure Required:
- Acquired through lungs or skin

Clinical Disease:
- Fever, stiff neck, headache, encephalitis

Diagnosis:
- Microscopy/histology (immunofluorescent antibodies available)
- NAAT

Treatment:
- Multi-drug regimens containing 4–5 agents such as amphotericin, fluconazole, albendazole and miltefosine
- (Additional alternative agents) voriconazole, flucytosine, pentamidine, azithromycin, clarithromycin, trimethoprim-sulfamethoxazole and sulfadiazine

G. *Blastocystis hominis*

Exposure Required:
- Oral ingestion of cysts in fecally contaminated water or food

Clinical Disease:
- Asymptomatic, diarrhea, abdominal discomfort

Diagnosis:
- Stool microscopy
- NAAT

Treatment:
- Metronidazole 500 mg PO TID for 5–10 days
- Tinidazole 2 g PO x1
- Paromomycin 500 mg PO TID for 7–10 days
- Trimethoprim-sulfamethoxazole (TMP-SMX) double strength 160/800 mg PO BID for 7 days
- Nitazoxanide 500 mg PO BID for 3 days

H. *Dientamoeba fragilis*

Exposure Required:
- Oral ingestion of fecally contaminated water or food

Clinical Disease:
- Diarrhea, nausea, abdominal discomfort

Diagnosis:
- Stool microscopy –issues with sensitivity
- NAAT

Treatment:
- Metronidazole 500 mg PO TID for 10 days
- Paromomycin 10 mg/kg PO TID for 7 days
- Iodoquinol 650 mg PO TID for 20 days
- Tetracycline 400 mg PO QID for 10 days
- Doxycycline 100 mg PO BID for 10 days

15. **Non-pathogenic Protozoa**

- Commensal flagellates – no treatment
- Commensal amoebae – no treatment

16. ***Enterobius vermicularis***

Exposure Required:
- Oral ingestion of embryonated eggs, autoinfection

Clinical Disease:
- Asymptomatic, perianal pruritus, vaginal irritation

Diagnosis:
- Microscopy - pinworm paddle or clear adhesive tape to collect eggs, not standard O+P, and direct visualization of adult females
- NAAT

Treatment:
Treatment of exposed contacts, all household members, and/or source patients, if not household members, is recommended and has been successful in both households and institutions.

- Pyrantel pamoate 11 mg/kg PO x1 then repeated 2–3 weeks later
- Albendazole 400 mg PO x1 then repeated 2–3 weeks later
- Mebendazole 100 mg PO x1 then repeated 2–3 weeks later
- (Inferior option) ivermectin 200 mcg/kg PO x 1 then repeated 2–3 weeks later

17. ***Trichuris trichiura***

Exposure Required:
- Oral ingestion of embryonated eggs

Clinical Disease:
- Asymptomatic, dysentery, tenesmus, weight loss, anemia, rectal prolapse

Diagnosis:
- Stool microscopy - standard O+P and direct visualization of adults on endoscopy
- NAAT

Treatment:
- (Preferred) mebendazole 100 mg PO BID x 3 days
- (Inferior option) albendazole 400 mg PO Q-day x 3 days
- (Inferior option) ivermectin 200 mcg/kg PO x 1

18. *Ascaris lumbricoides*

Exposure Required:
- Oral ingestion of embryonated eggs

Clinical Disease:
- Migratory phase – Löeffler's syndrome -pneumonitis, hepatomegaly, bronchospasm, eosinophilia
- Intestinal phase – asymptomatic, high burden can lead to obstruction, aberrant migration can lead to peritonitis or obstruction such as in hepatobiliary ascariasis

Diagnosis:
- Stool microscopy - standard O+P and direct visualization of adults on endoscopy
- NAAT

Treatment:
- Albendazole 400 mg PO x 1
- Mebendazole 500 mg PO x 1 or 100 mg PO BID for 3 days
- Pyrantel pamoate 11 mg/kg PO x1 (can be used during pregnancy)
- (Inferior option) ivermectin 200 mcg/kg PO x 1

19. **The Hookworms**

Necator americanus
Ancylostoma duodenale
Ancylostoma ceylanicum

Exposure Required:
- Infective L3 filariform larvae penetrate skin (usually through a hair follicle) *Ancyclostoma duodenale* larvae are also infective orally

Clinical Disease:
- Dermatitis-(during entry), pneumonia-(during migratory phase), abdominal pain-(can occur with heavy oral ingestion with eosinophilia 'Wakana disease'), chronic iron deficiency anemia, skin pigmentation change to yellow-green 'chlorosis'-(chronic disease)

Diagnosis:
- Stool microscopy - standard O+P and direct visualization of adults on endoscopy
- NAAT

Treatment:
Ivermectin has poor efficacy and is not recommended.
- (Preferred) albendazole 400 mg PO x 1
- Mebendazole 500 mg PO x 1 or 100 mg PO BID x 1 day
- Pyrantel pamoate 11 mg/kg PO Q-day x 3 days

20. *Strongyloides stercoralis*

Exposure Required:
- Infective L3 filariform larvae penetrate skin (usually through a hair follicle)

Clinical Disease:
- Asymptomatic, watery diarrhea, eosinophilia, dermatitis-('ground itch'), *larva currens* rash, periumbilical thumbprint purpura rash, with hyperinfection-bacterial sepsis and bacterial meningitis, with *S. fuelleborni* swollen belly syndrome

Diagnosis:
- Microscopy - stool O+P but larvae are seen not eggs, or histological examination of tissues
- Fecal culture - coproculture
- Serology
- NAAT

Treatment:
Control (not elimination)
- **Uncomplicated Infection:**
  - Ivermectin 200 mcg/kg/day given once and then repeated 2 weeks later (so for 60 kg adult 4 of the 3 mg tablets PO each time)
  - (Inferior alternative) albendazole 400 mg PO Q-day for 7-days
- **Disseminated Disease:**
  - Ivermectin 200 mcg/kg/day PO Q-day with duration determined by clinical response, some will add albendazole 400 mg PO Q-day if poor clinical response (Some patients have been successfully treated off label with subcutaneous dosing of veterinarian ivermectin preparations).

21. *Trichinella spiralis*

Exposure Required:
- Oral ingestion of raw or undercooked meats

Clinical Disease:
- Gastrointestinal phase – secretory diarrhea, abdominal pain, nausea, vomiting
- Parenteral phase – fever, myalgia, bilateral periorbital edema, petechial hemorrhages, leukocytosis, eosinophilia, can have CNS or cardiac involvement with meningoencephalitis or arrythmias

Diagnosis:
- Microscopy - histological examination of tissues after muscle biopsy
- Serology - ELISA with Western blot confirmation
- NAAT
- Supportive laboratory tests – muscle enzymes, such as creatine kinase, lactic dehydrogenase, and peripheral eosinophilia

Treatment:

- Albendazole 400 mg PO BID x 14 days
- Mebendazole 400 mg PO TID x 14 days
- Prednisone 30–60 mg Q-day for 14 days (add to regimen only if diagnosis secure)
- Antipyretics and analgesics

22. Lymphatic Filariae

Wuchereria bancrofti
Brugia malayi

Exposure Required:

- Bite from mosquito (wide variety of genera and species)

Clinical Disease:

- Asymptomatic – lymphatic dilation detectable only by ultrasound or other testing
- Acute lymphadenitis – fever, painful swelling of lymph nodes, secondary bacterial infections
- Elephantiasis – lymphedema of arms, legs, breasts, genitalia, secondary bacterial infections
- Tropical pulmonary eosinophilia – nocturnal asthma with dyspnea, fatigue, weight loss, and eosinophilia

Diagnosis:

- Microscopy - of blood smears during the night and can be concentrated
- Serology - ELISA with Western blot confirmation
- Antigen testing – circulating filarial antigen assay
- NAAT - research tool, no commercially available tests
- Ultrasound – lymphatic vessels and can detect filarial dance sign in the spermatic cord

Treatment:

It is critical that, prior to treatment, co-infection with *Loa loa* with a high *Loa loa* microfilarial load is ruled out, due to the risk of severe adverse events if treatment is given to such patients.

- **Antiparasitics:**
  - Diethylcarbamazine (DEC) 6 mg/kg/day x 12 days for a total of 72 mg/kg body weight
  - Doxycycline 100 mg PO BID for 6 weeks
  - Ivermectin as part of mass drug programs
  - Albendazole as part of mass drug programs
- **Surgery:** hydrocele drainage and lymphatic surgery
- **Complementary care:**
  - Lymphedema care
  - Treatment of wounds and secondary bacterial infections

23. *Onchocerca volvulus*

Exposure Required:

- Bite of the black fly (*Simulium* spp.)

Clinical Disease:

- Dermatitis – papular changes (can be extremely pruritic), lichenification (*sowda*), atrophy (hanging groin), depigmentation (leopard skin), reddish facial lesions (*erysipelas de la costa*)
- Lymphadenopathy – Africa (inguinal), Americas (head and neck)
- Ocular – keratitis, iritis, optic atrophy, optic neuritis, cataracts, chorioretinitis, blindness
- Nodding syndrome – not clearly caused by this parasite but presents with seizures, head nodding, periods of unresponsiveness, long-term disability

Diagnosis:

- Microscopy - bloodless skin snips, blood smears to rule out other infections
- Serology - ELISA with Western blot confirmation
- Mazzotti test - now modified test
- NAAT - research tool, no commercially available tests
- Ultrasound - evaluation of nodules

Treatment:

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 hrs of treatment. 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.

- **Endemic Area:**
  - Ivermectin 150 mcg/kg by mouth every 6 months for years
  - (Alternative) doxycycline 100 mg PO 1x/day x 6 weeks followed by ivermectin
- **Outside Endemic Area:**
  - Ivermectin 150 mcg/kg by mouth every 6 months for several years until asymptomatic
  - (Alternative) doxycycline 100 mg PO 1x/day x 6 weeks followed by ivermectin

24. *Loa loa*

Exposure Required:

- Bite from the deer fly (*Chrysops* spp.)

Clinical Disease:

- Asymptomatic, Calabar swellings, angioedema, localized swelling, worm migration across eye, cardiomyopathy, renal disease, encephalitis, lymphadenitis, eosinophilia, -serious adverse reactions when treated for other parasitic infections

Diagnosis:

- Microscopy - of blood smears during the middle of the day and can be concentrated
- Serology - ELISA with Western blot confirmation
- NAAT - research tool, no commercially available tests

Treatment:

It is critical that, prior to treatment, co-infection with *L. loa* with a high *L. loa* microfilarial load is ruled out, due to the risk of severe adverse events if treatment is given to such patients.

- **>2,500 microfilariae/ml**: If levels are greater than 2,500 mf/ml, then apheresis or treatment with albendazole 200 mg PO BID until loads <2,500 mf/ml.
- **<2,500 microfilariae/ml**:
    - Diethylcarbamazine (DEC)
        - CDC regimen: DEC 8 to 10 mg/kg/day in 3 divided doses for 21 days; patients with symptomatic loiasis and microfilarial loads ≥ 8,000 mf/mL should receive apheresis or treatment with albendazole prior to treatment with diethylcarbamazine (CDC 2015). For patients with microfilaria in the blood, some clinicians recommend the following dose-escalating regimen: 50 mg as a single dose on day 1; 50 mg 3 times daily on day 2; 100 mg 3 times daily on day 3; 9 mg/kg/day in 3 divided doses on day 4 to end of treatment course. Repeat courses of treatment may be needed to achieve cure.
        - World Health Organization recommendations: DEC 1 mg/kg as a single dose on day 1, with doubling of the dose on the next 2 days, then 6–9 mg/kg/day in divided doses 3 times daily for 18 days.
- **Surgery:** Adult worms in the eye can be removed surgically.
- **Albendazole:** Alternatives to DEC include albendazole 200 mg PO BID x 21 days that can effectively reduce the number of circulating microfilariae by acting directing 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.
- Chemoprophylaxis with weekly 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.

25. *Dracunculus medinensis*

Exposure Required:

- Oral ingestion of infected copepods

Clinical Disease:

- Cutaneous blisters and ulcers, allergic reactions and superinfection with failed attempts to remove worms, arthritis, contractures and scarring causing disability and absenteeism from work and school

Diagnosis:
- Direct visualization: locating head of the adult worm in the skin lesion
- Microscopy - identifying the larvae that are released into freshwater
- ELISA - availability limited
- Radiographs - calcifications corresponding to adult worms

Treatment:
- Slow mechanical extraction of about 1cm/day, pain control and treatment of any secondary bacterial infections

26. **Other Nematodes of Medical Importance**

A. ***Capillaria hepatica***

Exposure Required:
- Oral ingestion of embryonated eggs

Clinical Disease:
- Asymptomatic, liver failure, abdominal lymphadenopathy, eosinophilia

Diagnosis:
- Histology - after liver biopsy or at autopsy
- Serological testing - ELISA and indirect fluorescent antibody test (IFA), high sensitivity and specificity but not widely available

Treatment:
- Combination therapy has been used successfully with combinations of albendazole, thiabendazole, disophenol (2-6-diiodo-4-nitrophenol) and prednisone

B. ***Capillaria philippinensis***

Exposure Required:
- Oral ingestion of raw or undercooked freshwater fish or crustaceans

Clinical Disease:
- Diarrhea

Diagnosis:
- Stool microscopy - standard O+P with direct visualization of eggs or larvae in stool or detecting the adults on small bowel biopsy of the small intestinal wall

Treatment:
- Albendazole 400 mg PO Q-day x 30 days
- Mebendazole 200 mg PO BID x 20–30 days

C. *Dirofilaria immitis*

Exposure Required:
- Bite of infected mosquito

Clinical Disease:
- Coin lesion in lung

Diagnosis:
- Histology
- Radiographs - coin lesion in lungs

Treatment:
No known effective therapies in humans

D. *Mansonella ozzardi*

Exposure Required:
- Bite of black flies and biting midges

Clinical Disease:
- Asymptomatic, urticaria, lymphadenopathy, chronic arthritis, eosinophilia

Diagnosis:
- Microscopy - visualization of microfilariae in a blood smear, sensitivity increased with concentration techniques
- ELISA - based on crude antigen preparations but with limited specificity
- PCR - developed and available through the National Institutes of Health

Treatment:
- Ivermectin 200 mcg/kg/day PO x1

E. *Mansonella perstans*

Exposure Required:
- Bite from biting midge

Clinical Disease:
- Asymptomatic, painless conjunctival nodules, eyelid swelling, angioedema, (called the Ugandan or Kampala eye worm)

Diagnosis:
- Microscopy - visualization of microfilariae in a blood smear, sensitivity increased with concentration techniques
- ELISA - based on crude antigen preparations but with limited specificity
- PCR - developed and available through the National Institutes of Health

Treatment:
Considered one of the most challenging filarial infections to treat.
- Combination therapy has been successful
- Doxycycline has been successfully used on strains from Mozambique and the Democratic Republic of Congo that harbor the endosymbiont Wolbachia

F. *Mansonella streptocerca*

Exposure Required:
- Biting midges

Clinical Disease:
- Pruritic dermatitis, hypopigmented macules

Diagnosis:
- Microscopy - visualization of microfilariae in a blood smear, sensitivity increased with concentration techniques
- ELISA - based on crude antigen preparations but with limited specificity
- PCR - developed and available through the National Institutes of Health

Treatment:
- Diethylcarbamazine (DEC) 2 mg/kg/day TID for 12 days
- Ivermectin 150 mcg/kg/day PO x1 can reduce microfilarial levels

G. *Oesophagostomum bifurcum*

Exposure Required:
- Oral ingestion of infective larvae

Clinical Disease:
- Asymptomatic, abdominal nodules or masses, abdominal pain

Diagnosis:
- Stool microscopy - standard O+P with direct visualization of eggs (cannot be visually distinguished from hookworm eggs)
- Coproculture - allowing eggs to develop to third stage larvae (difficult and time consuming)
- Histology of biopsied nodules showing larval or adult forms
- Imaging - Ultrasound
- NAAT - PCR/Multiplex

Treatment:
- Pyrantel pamoate 11mg/kg PO x1
- Albendazole 400 mg PO x1

H. *Ternidens diminutus*

Exposure Required:
- Oral ingestion of infective larvae

Clinical Disease:
- Colonic ulcerations, nodular lesions, abdominal mass

Diagnosis:
- Stool microscopy - standard O+P with direct visualization of eggs (can not be visually distinguished from hookworm eggs)

Treatment:
- Pyrantel pamoate 11 mg/kg PO x1
- Albendazole 400 mg PO x1

27. Aberrant Nematode Infections

A. Cutaneous Larva Migrans

Exposure Required:
- Infective larvae penetrate unbroken skin

Clinical Disease:
- Serpiginous lesions, pruritus, secondary bacterial infections

Diagnosis:
- Physical findings: visualization of serpiginous lesions
- Dermoscopy - translucent brown areas and red-dotted vessels

Treatment:
- (Preferred) Ivermectin 200 mcg/kg PO Q-day x 1–2 days
- (Alternative) Albendazole 400 mg PO Q-day x 3–7 days
- Topical thiabendazole 15% applied daily for 5 days
- Topical albendazole ointment 10% applied TID x 10 days

B. Visceral Larva Migrans

Exposure Required:
- Oral ingestion of embryonated eggs

Clinical Disease:
- Tissue damage, cognitive defects, hypersensitivity responses, eosinophilia

Diagnosis:
- Serology - ELISA
- NAAT - research tool, no commercially available tests
- Ophthalmological exam for ocular larva migrans (OLM)
- Imaging - CT, MRI, Ultrasound

Treatment:
- Steroids for severe or CNS reactions
- Albendazole 400 mg PO BID x 5 days

C. **Ocular Larva Migrans**

Exposure Required:
- Oral ingestion of embryonated eggs

Clinical Disease:
- Ocular granulomas, visual disturbance, blindness

Diagnosis:
- Serology - ELISA

Treatment:
- Steroids for some ophthalmic or CNS reactions
- Albendazole 400 mg PO BID x 5 days
- Surgery (vitrectomy)

D. ***Baylisascaris procyonis***

Exposure Required:
- Oral ingestion of embryonated eggs

Clinical Disease:
- Nausea, fatigue, hepatomegaly, loss of coordination and muscle control, eosinophilic meningitis, ocular disease, encephalitis, coma

Diagnosis:
- Serology - recombinant protein-based ELISA

Treatment:
- Albendazole 25–50 mg/kg/day in divided doses x 20 days, with concomitant steroids

E. ***Angiostrongylus cantonensis/costaricensis***

Exposure Required:
- Oral ingestion of infective larvae

Clinical Disease:
- *A. cantonensis* - eosinophilic meningoencephalitis, fever, headache, painful paresthesias
- *A. costaricensis* – abdominal pain, fever, nausea, vomiting

Diagnosis:
- CSF examination for eosinophilia
- PCR of CSF fluid
- Imaging - MRI may reveal areas of enhancement in characteristic patterns
- Serology - ELISA (not widely available and older assays cross react with gnathostomiasis)

Treatment:
- Analgesics, repeated lumbar punctures, steroids and albendazole are used but optimal treatment is not defined

F. *Gnathostoma spinigerum*

Exposure Required:
- Ingestion of infective larvae in fish, snakes, and birds

Clinical Disease:
- Asymptomatic, cutaneous larva migrans, subcutaneous swellings, eosinophilic meningitis, painful paresthesias

Diagnosis:
- CSF examination for eosinophilia
- Imaging - MRI may reveal areas of enhancement in characteristic patterns and CT may demonstrate areas of hemorrhage
- Serology - ELISA (not widely available and older assays cross react with *Angiostrongylus cantonensis*)

Treatment:
- Albendazole 400 mg PO Q-day x 21 days
- Ivermectin 200 mcg/kg PO Q-day x 2 days
- Repeated lumbar punctures to reduce opening pressure, steroids

G. *Anisakiasis*

Exposure Required:
- Oral ingestion of infective larvae in raw or undercooked saltwater fish or squid

Clinical Disease:
- Abdominal pain, nausea, vomiting, diarrhea, fever

Diagnosis:
- Direct visualization of parasites by endoscopy or in vomit
- Serology - ELISA
- PCR - not commercially available outside research settings

Treatment:
- Physical removal of the parasite prior to penetration
- Surgery after penetration
- Albendazole 400 mg PO BID for 3–21 days but optimal therapy is not defined

28. *Taenia saginata*

Exposure Required:
- Ingestion of raw or undercooked beef containing cysticerci

Clinical Disease:
- Asymptomatic, proglottids noted in stool or clothing

Diagnosis:
- Microscopy:
    - Gravid proglottids can be fixed in 10% formaldehyde solution, and the uterus injected with India ink, with the aid of a 26-gauge needle or stained with hematoxylin-eosin staining techniques. *T. saginata* proglottids have 12 or more branches on either side of the uterus.
    - Eggs of *T. saginata* are occasionally found in stool, since most proglottids usually pass out of the host intact. If an egg is seen on stool examination, the species cannot be determined on visual microscopy 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.
    - Paddle Test/Sticky Tape Test is an additional diagnostically relevant test (see: diagnosis for *Enterobius vermicularis*). When proglottids migrate out of the anus, they express eggs that remain on the perineum.
- NAAT – PCR/ loop-mediated isothermal amplification (LAMP)/Multiplex
- Antigen detection (coproantigens) – used on stool samples

Treatment:
- Praziquantel 5–10 mg/kg PO x1 (600 mg tablets)
- Niclosamide 2 g PO x1 (not commercially available in the United States)

29. *Taenia solium*

Intestinal

Exposure Required:
- Ingestion of raw or undercooked pork containing cysticerci

Clinical Disease:
- Asymptomatic, proglottids noted in stool or clothing

Diagnosis:
- Microscopy:
    - Gravid proglottids can be fixed in 10% formaldehyde solution, and the uterus injected with India ink, with the aid of a 26-gauge needle or stained with hematoxylin-eosin staining techniques. *T. solium* proglottids have less than 12 branches on either side of the uterus.

    - Eggs of *T. solium* are occasionally found in stool, since most proglottids usually pass out of the host intact. If an egg is seen on stool examination, the species cannot be determined on visual microscopy 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 and *T. solium* eggs have a shell that is not acid-fast.
    - Paddle Test/Sticky Tape Test is an additional diagnostically relevant test (see: diagnosis for *Enterobius vermicularis*). When proglottids migrate out of the anus, they express eggs that remain on the perineum.
- NAAT - PCR/ loop-mediated isothermal amplification (LAMP)/Multiplex
- Antigen detection (coproantigens) - used on stool samples

Treatment:
- Praziquantel 5–10 mg/kg PO x1 (600 mg tablets)
- Niclosamide 2 g PO x1 (not commercially available in the United States)

Extra-intestinal (Cysticercosis and Neurocysticercosis)

Exposure Required:
- Oral ingestion of embryonated eggs

Clinical Disease:
- Extraneural – subcutaneous (discrete swellings that go on to become tender), intramuscular (asymptomatic, cysts in muscles, calcifications)
- Neurocysticercosis – intraparenchymal/extraparenchymal (space occupying symptoms, headaches, seizures, hydrocephalus, focal neurological abnormalities)

Diagnosis:
- Imaging - recommend that patients undergo both MRI and CT for CNS disease, plain radiographs can also show peripheral calcified disease
- Serology - Enzyme-linked immune-transfer blot, rather than crude antigen assays
- NAAT - PCR/ loop mediated isothermal amplification (LAMP) /Multiplex

Treatment:
- Monotherapy for 1–2 viable cysts - albendazole 7.5 mg/kg PO BID for 10 days (200 mg tablets)
- Combination therapy for > 2 viable cysts - albendazole 7.5 mg/kg PO BID for 10 days (200 mg tablets) and praziquantel 5 mg/kg PO TID for 10 days (600 mg tablets)
- Corticosteroids (recommended for all CNS disease when using antiparasitics) - prednisone 1 mg/kg PO Q-day x 5–10 days then tapered or dexamethasone 10 mg IV x1 then 4 mg IV q6 hrs x 5–10 days then tapered. For acute encephalitis steroids alone without antiparasitics are recommended.
- Antiepileptic therapy - recommended for all patients having seizures
- Mechanical therapy - for extraparenchymal neurocysticercosis surgical therapy and ventriculoperitoneal shunting may be required.

30. *Diphyllobothrium latum*

Exposure Required:
- Oral ingestion of infective larvae from eating raw or undercooked freshwater or andromous fish

Clinical Disease:
- Asymptomatic, watery diarrhea, fatigue, B12 deficiency

Diagnosis:
- Microscopy:
    - Gravid proglottids can be fixed in 10% formaldehyde solution, and stained with hematoxylin-eosin staining techniques. These proglottids are wider than they are long (most proglottids do not pass out of the host intact).
    - Eggs can be found in stool (most proglottids do not pass out of the host intact)
- NAAT

Treatment:
- Praziquantel 5–10 mg/kg PO x1 (600 mg tablets)
- Niclosamide 2 g PO x1 (not commercially available in the United States)

31. **Other Tapeworms of Medical Importance**

A. *Hymenolepis nana*

Exposure Required:
- Oral ingestion of infective larvae along with infected insect or oral ingestion of embryonated eggs

Clinical Disease:
- Asymptomatic, rarely diarrhea

Diagnosis:
- Standard detection of eggs on stool O+P

Treatment:
- Praziquantel 5–10 mg/kg PO x1 (600 mg tablets)
- (Inferior alternative) nitazoxanide 500 mg PO BID x 3 days

B. *Hymenolepis diminuta*

Exposure Required:
- Oral ingestion of infective larvae along with infected insect

Clinical Disease:
- Asymptomatic

Diagnosis:
- Standard detection of eggs on stool O+P

Treatment:
- Praziquantel 5–10 mg/kg PO x1 (600 mg tablets)
- (Inferior alternative) nitazoxanide 500 mg PO BID x 3 days

C. *Dipylidium caninum*

Exposure Required:
- Oral ingestion of infected adult fleas

Clinical Disease:
- Asymptomatic

Diagnosis:
- Standard detection of eggs on stool O+P

Treatment:
- Praziquantel 5–10 mg/kg PO x1 (600 mg tablets)
- (Inferior alternative) nitazoxanide 500 mg PO BID x 3 days

32. Juvenile Tapeworm Infections of Humans

A. *Echinococcus granulosus*

Exposure Required:
- Oral ingestion of embryonated eggs

Clinical Disease:
- Liver cysts, lung cysts, cysts in any organ, anaphylactic reactions with cyst rupture

Diagnosis:
- Imaging: cysts can be visualized with CT, MRI and ultrasound
- Microscopy: examination of cyst contents and cysts themselves
- Serological testing: sensitivities vary by cyst stage
- NAAT

Treatment:

- Based on Stage CE1–CE5:
  - CE1
    - < 5cm - Albendazole 400 mg PO BID
    - > 5cm - Albendazole 400 mg PO BID and puncture, aspiration injection, re-aspiration (PAIR)
  - CE3a
    - < 5cm - Albendazole 400 mg PO BID
    - > 5cm - Albendazole 400 mg PO BID and PAIR
  - CE2 - Albendazole 40 0 mg PO BID and large bore percutaneous treatment, (PAIR is contraindicated)
  - CE3b - Albendazole 400 mg PO BID and large bore percutaneous treatment, (PAIR is contraindicated)
  - CE4 & CE5 - observation with imaging every 6 months, (PAIR is contraindicated)
  - Surgery - indicated for cysts >10 cm, ruptured cysts, extra-hepatic disease, very complex cysts (many daughter cells) and cysts that have formed fistula.

B. *Echinococcus multilocularis*

Exposure Required:

- Oral ingestion of embryonated eggs

Clinical Disease:

- Proliferative membranes primarily in the liver leading to hepatic failure, abdominal pain, weight loss, fatigue

Diagnosis:

- Imaging - lesions can be visualized with CT, MRI and ultrasound
- Microscopy - histology of directed biopsy specimens
- Serological testing - sensitive and specific enough to distinguish from *E. granulosus*
- NAAT

Treatment:

- Surgery is the primary approach when possible
- Albendazole 400 mg PO BID suggested minimum of 2 years but indefinitely if not amenable to surgery

C. *Mesocestoides* spp.

Exposure Required:

- Oral ingestion of infective larvae in under cooked bird, snake, lizard, amphibian or mammalian carnivore

Clinical Disease:

- Mild abdominal discomfort, nausea, diarrhea, vomiting

Diagnosis:

- Detection of eggs on stool O+P

Treatment:
- Praziquantel 5–10 mg/kg PO x1 (600 mg tablets)
- (Inferior alternative) nitazoxanide 500 mg PO BID x 3 days

D. *Spirometra* spp.

Exposure Required:
- Oral ingestion of infective larvae in undercooked meat or exposure to larvae from poultice that then invade through wound or mucous membrane

Clinical Disease:
- Asymptomatic, orbital edema, neurological complications

Diagnosis:
- Identification of the parasite after removal or biopsy

Treatment:
- Primarily Surgical Management

E. *Taenia* spp. *(*other than *T. saginata* and *T. solium)*

Exposure Required:
- Oral ingestion of embryonated eggs

Clinical Disease:
- Mass effect in organ invaded, may invade CNS (brain, eyes, spinal cord)

Diagnosis:
- Identification of the parasite after removal or biopsy

Treatment:
- Primarily surgical management

33. The Schistosomes

Schistosoma mansoni
Schistosoma japonicum
Schistosoma haematobium
Schistosoma mekongi
Schistosoma intercalatum

Exposure Required:
- Infective cercariae enter skin (usually through a hair follicle)

Clinical Disease:
- **Acute** – 'Katayama fever' hepatomegaly, splenomegaly, lymphadenopathy, fever, myalgias, cough, headache, eosinophilia
- **Chronic** – abdominal pain, diarrhea, hepatomegaly, splenomegaly, hematuria, vaginal symptoms in female genital schistosomiasis (FGS), CNS - (focal transverse myelitis, encephalitis)

Diagnosis:

- Microscopy: detection of schistosome eggs in stool or urine. Detection of eggs in an unfixed rectal snip/biopsy
- Antigen detection: two schistosome glycoprotein antigens known as CCA and CAA circulate in the bloodstream of acutely infected patients and can be detected with certain assays
- Serology: antibodies develop 6–12 weeks after exposure and tend to become positive before eggs are evident in urine or stool. (ELISA, indirect hemagglutination assay (IHA), radioimmunoassay, complement fixation, Western blot)
- Imaging: portable ultrasound imaging has been shown to be clinically useful in the diagnosis of schistosomiasis

Treatment:

Detection of viable eggs 6 weeks after treatment warrants retreatment

- **Acute Infection**
  - Prednisone 40 mg PO Q-day x 5 days
  - Praziquantel 20 mg/kg PO TID x 1 day (6 weeks after exposure and when acute symptoms have resolved) then repeated 6 weeks later (600 mg tablets)
- **Chronic Infection**
  - Praziquantel 20 mg/kg PO TID x 1 day (8 weeks after exposure and when acute symptoms have resolved) (600 mg tablets)
- **CNS Schistosomiasis**
  - Prednisone 1mg/kg PO Q-day started immediately with duration based on response and clinical course

34. *Clonorchis sinensis* and *Opisthorchis* spp.

Exposure Required:

- Oral ingestion of raw or undercooked freshwater fish containing metacercariae

Clinical Disease:

- Asymptomatic, right upper quadrant abdominal pain, nausea, diarrhea, headache, hepatomegaly, eosinophilia

Diagnosis:

- Microscopy - after 4 weeks eggs will be released into feces. The sensitivity can be improved with NAAT (PCR and loop-mediated isothermal amplification ([LAMP])
- Endoscopy - endoscopic retrograde cholangiopancreatography (ERCP) may allow visualization of flukes
- Serology - ELISA with confirmatory Western blot is available
- Imaging - the presence of flukes in the biliary tract may also be observed using ultrasound, CT, MRI and cholangiography

Treatment:

- **Antiparasitics**
  - Praziquantel 25 mg/kg PO TID x 2 days or praziquantel 40 mg/kg PO x1 for light infections (600 mg tablets)
  - Albendazole 10 mg/kg PO Q-day x 7 days
  - Mebendazole 30 mg/kg PO Q-day x 30 days
- **Mechanical Interventions**
  - Surgery, biliary drainage and broad-spectrum antibiotics may be required in certain cases

35. *Fasciola hepatica*

Exposure Required:

- Oral ingestion of metacercariae on watercress or other littoral plants

Clinical Disease:

- Early phase – fever, right upper quadrant abdominal pain, malaise, headache, eosinophilia
- Chronic – dull right upper quadrant pain, biliary obstruction

Diagnosis:

- Serology - serological tests become positive early in disease during migration through the liver parenchyma
- Antigen tests - available with high sensitivity and specificity
- Microscopy - after 4 months eggs will start to be released into feces
- Endoscopy - endoscopic retrograde cholangiopancreatography (ERCP) may allow visualization of flukes
- Imaging - the presence of linear migratory tracts and adult flukes may also be observed using ultrasound, CT, MRI and cholangiography

Treatment:

In the United States Triclabendazole can be obtained through the CDC.

- Triclabendazole 10 mg/kg PO x1 and then in severe infections may be repeated 12–24 hrs after the first dose. Successfully treated patients will develop negative serologies 6–12 months after clearing their parasites.
- (Inferior alternative) Nitazoxanide 500 mg PO BID for 7 days

36. *Paragonimus westermani / P. kellicotti*

Exposure Required:

- Oral ingestion of metacercariae on raw or undercooked crab or crustaceans

Clinical Disease:

- Acute - asymptomatic, diarrhea, fever, chest pain, fatigue, urticaria, epigastric pain, eosinophilia
- Late – fever, chills, cough, dyspnea, blood tinged sputum, hemoptysis, pulmonary infiltrates, pulmonary lesions, CNS-more common with *P. kellicotti*

Diagnosis:
- Serology - serological tests are important in early disease before egg production occurs which can take 8–12 weeks
- Microscopy - late-stage disease is diagnosed by microscopic identification of eggs in the sputum, bronchoalveolar lavage fluid, and, more rarely, in stool
- Imaging - ultrasound, X-ray examinations, CT, MRI and fluorodeoxyglucose-positron emission tomography (FDG-PET)

Treatment:
- Praziquantel 25 mg/kg PO TID for 3 days (600 mg tablets)
- Triclabendazole 10 mg/kg PO x1

37. Other Trematodes of Medical Importance

A. *Fasciolopsis buski*

Exposure Required:
- Oral ingestion of metacercariae on husks of seeds of littoral plants such as water chestnuts

Clinical Disease:
- Asymptomatic, diarrhea, vomiting, nausea, fever, intestinal hemorrhage, abdominal pain, eosinophilia

Diagnosis:
- Microscopy - identification of eggs or flukes in stool or vomit

Treatment:
- Praziquantel 25 mg/kg PO TID x 1 day (600 mg tablets)

B. *Echinostoma* spp.

Exposure Required:
- Oral ingestion of metacercariae from ingesting various species of snails, tadpoles, or freshwater fish

Clinical Disease:
- Diarrhea, nausea, vomiting, abdominal pain, fever

Diagnosis:
- Microscopy - identification of eggs in stool or in some cases flukes recovered from endoscopy

Treatment:
- Praziquantel 25 mg/kg PO x 1 day (600 mg tablets)

C. *Heterophyes heterophyes/Metagonimus yokogawai*

Exposure Required:
- Oral ingestion of metacercariae from ingesting certain freshwater fish

Clinical Disease:
- Epigastric pain, fatigue, diarrhea, weight loss, malaise, belching, nausea, headache, vomiting

Diagnosis:
- Microscopy - identification of eggs in stool
- NAAT – only available in research settings

Treatment:
- Praziquantel 25 mg/kg PO TID x 1 day (600 mg tablets)

D. *Nanophyetus salmincola*

Exposure Required:
- Ingestion of raw or undercooked salmon containing metacercariae

Clinical Disease:
- Diarrhea, nausea, vomiting, anorexia, eosinophilia

Diagnosis:
- Microscopy: identification of eggs in stool

Treatment:
- Praziquantel 25 mg/kg PO TID x 1 day (600 mg tablets)
- Niclosamide 2 g PO x1 (not commercially available in the United States)

38. **The Insects**

A. **Myiasis-Causing Flies: Calliphoridae, Cuterebridae, and Sarcophagidae**

Exposure Required:
- Larvae penetrate intact skin or wounds

Clinical Disease:
- Abscess like swellings with openings, maggots visible in wounds

Diagnosis:
- Visualization of living or dead maggots, but suggested by abscess like lesions with small central opening

Treatment:
- Surgical removal is the primary treatment
- Alternatively, if the opening is blocked with a substance such as petroleum jelly, that blocks access to oxygen, they can be forced to crawl to the surface and be removed

B. **Anaplura: Sucking Lice**

Exposure Required:

- Direct contact with an infected individual for hair lice / physical contact and clothing for body lice

Clinical Disease:

- Pruritis

Diagnosis:

- Visualization of lice or eggs in the hair or seams of garments. The wet combing technique increases sensitivity for detection of lice attached to hair.

Treatment:

- **For body lice:**
  - Manual removal of lice using the wet combing technique can be performed
  - Topical application of pediculicides (for age >2 months of age - permethrin (1%) cream rinse leave on hair for 10 minutes, rinse off and repeat 9 days later, > 6 months of age benzyl alcohol (5%) lotion leave on hair for 10 minutes, rinse off and repeat 7 days later, > 6 months of age ivermectin (0.5%) topical lotion leave on hair for 10 minutes, rinse off, > 2 years of age pyrethrins (0.33%) with piperonyl butoxide (4%) lotion apply to dry hair and leave on hair for 10 minutes, rinse off and repeat 9 days later.
  - (Alternative) > 6 years of age – malathion (0.5%) lotion leave on hair for 8–12 hrs then wash off, may repeat in 9 days
  - Oral treatment with ivermectin 200–400 mcg/kg PO x1 (3 mg tablets so ~5–6 tablets for a 70 kg adult) with a second treatment on day 8 if live lice detected
- **For body lice:**
  - Thoroughly bathe patient and wash clothing in heated water >149 ° F, occasionally topical therapy with permethrin (5%) cream to entire body and left on for 8–10 hrs. Low potency topical steroids may be used for symptomatic relief.
  - Oral treatment with ivermectin 200–400 mcg/kg PO x1 may have a transient impact on body lice infestation
- **For pubic lice:**
  - Manual removal of lice using the wet combing technique can be performed
  - Topical application of pediculicides (for age >2 months of age – permethrin (1%) cream rinse, leave on affected areas for 10 minutes, pyrethrins (0.33%) with piperonyl butoxide (4%) lotion apply to affected areas and leave on hair for 10 minutes
  - Oral treatment with ivermectin 250 mcg/kg PO x1 (3mg tablets so ~5–6 tablets for a 70 kg adult) repeated 1–2 weeks later

39. The Arachnids

A. Ticks

Exposure Required:
- Exposure to ticks

Clinical Disease:
- Various manifestations for different tick-borne diseases, imbedded tick

Diagnosis:
- Visualization of the tick

Treatment:
- Gently but firmly pulling the tick away from its point of attachment so the entire tick, including its mouthparts, is removed. It is recommended that chemical means be avoided and burning or smothering ticks is not attempted.

B. Scabies: *Sarcoptes scabiei*

Exposure Required:
- Direct contact with an infected individual

Clinical Disease:
- Itching, skin rash

Diagnosis:
- Skin scraping and microscopic identification of scabies mites, eggs or feces. Dermoscopy can be helpful to visualize burrows, mites and identification of 'delta wing' sign and also to direct scrapings.

Treatment:
- Topical application of permethrin (1%) cream leave on entire body including under the nails for 8–14 hrs, rinse off and repeat 1–2 weeks later if needed, 30 g typically required to cover entire body
- Oral treatment with ivermectin 200 mcg/kg PO x1 (3mg tablets so ~5 tablets for a 70 kg adult) with a second treatment on day 8 if live lice detected

Important note: A clinician experienced in the treatment of these diseases should guide all diagnostic modality and treatment selections. This appendix serves as a quick reference guide, but we recommend review of this material with sources of updated treatment and diagnosis such as the CDC before making diagnostic or treatment decisions. We also recommend that exact dosing, side effects, drug interactions and consideration of patient allergies be verified and considered.

Exposures

Exposure/Infection	
Oral (typically contaminated food or water)	Amoebiasis, Angiostrongyliasis, Anisakiasis, Ascariasis Balantidiasis, Baylisascariasis, Blastocystosis Clonorchiasis, Cryptosporidiosis, Cyclosporiasis, Cysticercosis, Cystoisosporiasis Dientamoebiasis, Diphyllobothriasis, Dracunculiasis Echinococcosis Fascioliasis Giardiasis, Gnathostomiasis Hepatic capillariasis Intestinal capillariasis Mesocestoidiasis Oesophagostomiasis Paragonimiasis, Pin worms Sparganosis Taeniasis-meat, Ternidensiasis, Toxoplasmosis, Trichinellosis, Trichuriasis VLM/OLM
Vector (flies, mosquitos, insect feces, ticks, midges)	African Trypanosomiasis (Tsetse fly), American Trypanosomiasis (Reduviid bug feces) Babesiosis (tick bite) Dirofilariasis (mosquito) Leishmaniasis (sand fly), Loiasis (deer fly), Lymphatic filariasis (mosquito) Malaria (mosquito), Mansonellosis (black flies/midges) Onchocerciasis (black fly)
Contact (mucous membranes, intact skin, wounds)	Acanthamoebiasis, American Trypanosomiasis Hookworms Larva Migrans, Lice (includes human-human contact) Myiasis Naeglariasis Scabies (includes human-human contact), Schistosomiasis, Sparganosis, Strongyloidiasis Ticks, Trichomoniasis (includes human-human contact)
Respiratory (inhalation)	Acanthamoebiasis Balamuthiasis Toxoplasmosis

Diagnostic and Laboratory Abnormalities

Diagnostics and Laboratory Abnormalities	
Anemia	Babesiosis, Hookworms, Malaria, Trichuriasis
Arrhythmias	American Trypanosomiasis, Trichinellosis
Eosinopenia	Malaria
Eosinophilia	Angiostrongyliasis, Ascariasis-migration, Clonorchiasis, Cryptosporidiosis, Dientamoebiasis, Hepatic capillariasis, Hookworm-migration, Fascioliasis, Loiasis, Lymphatic filariasis-TPE, Mansonellosis, Paragonimiasis, Schistosomiasis, Strongyloidiasis, Trichinellosis, VLM
Eosinophils in CNS	Angiostrongyliasis, Baylisascariasis, Gnathostomiasis, Schistosomiasis
Hepatomegaly	Ascariasis, Baylisascariasis, Clonorchiasis, Leishmaniasis-visceral, Schistosomiasis, Toxoplasmosis
Lesions in CNS	Amoebiasis - rare, Baylisascariasis, Cysticercosis, Toxoplasmosis, Paragonimiasis
Lesion in Liver	Amoebiasis, Echinococcosis, Fascioliasis
Lesion in Lungs	Amoebiasis – rare, Dirofilariasis, Echinococcosis, Paragonimiasis
Lesion in Spleen	Echinococcosis
Leukocytosis	Trichinellosis
Leukopenia	Malaria, Babesiosis
Liver Failure	Hepatic capillariasis

Symptoms

Abdominal Pain\ GI symptoms	Giardiasis, American Trypanosomiasis, Malaria, Cryptosporidiosis, Amoebiasis, Balantidiasis, Cystoisosporiasis, Naeglariasis, Blastocystosis, Dientamoebiasis, Trichuriasis-tenesmus, Ascariasis, Strongyloidiasis, Trichinellosis, Hepatic capillariasis, Oesophagostomiasis, Ternidensiasis, Baylisascariasis, Angiostrongyliasis-costaricensis, Anisakiasis, Mesocestoidiasis, Schistosomiasis, Clonorchiasis, Fascioliasis, Paragonimiasis
Anal Irritation	Pin worms
Diarrhea	Giardiasis, Cryptosporidiosis, Amoebiasis, Balantidiasis, Cystoisosporiasis, Cyclosporiasis, Blastocystosis, Dientamoebiasis, Trichuriasis, Strongyloidiasis, Trichinellosis, Intestinal capillariasis, Anisakiasis, Mesocestoidiasis, Schistosomiasis, Paragonimiasis
Pulmonary symptoms	American Trypanosomiasis, Malaria, Toxoplasmosis, Ascariasis, Hookworms, Lymphatic filariasis-TPE, Echinococcosis, Schistosomiasis
Neurological symptoms	African Trypanosomiasis, American Trypanosomiasis, Toxoplasmosis, Naeglariasis, Acanthamoebiasis, Balamuthiasis, Trichinellosis, Loiasis, Visceral larva migrans-cognitive defects, Baylisascariasis, Angiostrongyliasis, Sparganosis, Schistosomiasis
Facial Swelling	African Trypanosomiasis, American Trypanosomiasis, Trichinellosis, Sparganosis
Fever or Chills	Leishmaniasis-visceral, American Trypanosomiasis, Malaria, Toxoplasmosis, Balantidiasis, Babesiosis, Naeglariasis, Acanthamoebiasis, Balamuthiasis, Trichinellosis, Lymphatic filariasis, Angiostrongyliasis, Anisakiasis, Schistosomiasis, Fascioliasis, Paragonimiasis
Headache	African Trypanosomiasis, Babesiosis, Naeglariasis, Acanthamoebiasis, Balamuthiasis, Angiostrongyliasis, Cysticercosis, Clonorchiasis, Fascioliasis
Lymphadenopathy	African Trypanosomiasis, Toxoplasmosis, Onchocerciasis, Hepatic capillariasis-abdominal, Mansonellosis-ozzardi
Lymphedema\ Edema	American Trypanosomiasis, Lymphatic filariasis, Loiasis, Trichinellosis-bilateral periorbital edema
Malaise\Fatigue	American Trypanosomiasis, Toxoplasmosis, Balantidiasis, Babesiosis, Cystoisosporiasis, Lymphatic filariasis-TPE, Baylisascariasis, Diphyllobothriasis
Myalgia	Trichinellosis, Cysticercosis-localized, Schistosomiasis
Nodules/ Swelling	Onchocerciasis, Loiasis, Mansonellosis-perstans, Gnathostomiasis, Cysticercosis
Ocular symptoms	Acanthamoebiasis, Onchocerciasis, Loiasis, Mansonellosis-perstans, Ocular larva migrans, Baylisascariasis
Pruritis	African Trypanosomiasis, Mansonellosis, Onchocerciasis
Seizures	African Trypanosomiasis, Toxoplasmosis, Cysticercosis
Skin changes\ Rash	Leishmaniasis-visceral, Hookworms, Onchocerciasis, Mansonellosis, Larva Migrans
Ulcers	Leishmaniasis-cutaneous, African Trypanosomiasis, Dracunculiasis
Vaginal symptoms	Trichomoniasis, Schistosomiasis

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-ˈnȯr-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-ˈnȯr-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	lake, pay, eight, pain
æ	a	short a	fat, pat, dad, cat
i	ē	long e	bee, keep, eat, heat
ɛ	e	short e	bed, pet, pepper, desk, fetch
ɑɪ	ī	long i	hike, pie, by, high, try
ɪ	i	short i	fit, pit, it, dig, drink, system
oʊ	ō	long o	home, toe, dough, oh, boat
ɑ	‘ä	short o	mop, pot, hot, body
ʌ	‘ə	short u	but, bud, cup, flood, son
ʊ	u̇	other u	pull, wolf, wood, good, put, could
u	ü	oo sound	spoon, two, shoot, influence, move, do
ɔ	ȯ	aw sound	paw, walk, talk, saw, Paul
ɔɪ	ȯi	oi sound	join, coin, toy
aʊ	au̇	ow sound	how, vow, bow, house
a:	ä	ar sound	car, dark, park, farther
ɔr	u̇r	or sound	store, poor, tour
ɛr	‘e	air sound	pair, care, there
ɝ	ər	er sound	work, were, bird, dirt, nurse, stir, courage
ə	ə	*schwa	about, ago, famous, ocean
b	b	b sound	baby
ʧ	ch	ch sound	cheese
d	d	d sound	dig
f	f	f sound	face
g	g	g sound	gift
h	h	h sound	hat
ʤ	j	j sound	jump
k	k	k sound	cat
l	l	l sound	like
m	m	m sound	me
n	n	n sound	not
ŋ	ng	ng sound	sing
p	p	p sound	pig
r	r	r sound	red
s	s	s sound	some
ʃ	sh	sh sound	she
t	t	t sound	time
θ	th	unvoiced th	think
ð	*th*	voiced th	them
v	v	v sound	very
w	w	w sound	water
y	y	y sound	yes
z	z	z sound	zip
ʒ	zh	zh sound	usual

*The symbol [ə] is called a “schwa”. It is only used to represent unstressed syllables.

Name	Pronunciation Guide	American IPA
Acanthamoeba	ah-KAN-thah-MEE-bah	ə-ˌkanth-ə-ˈmē-bə
Adenocephalus	add-no-SEF-a-lus	ə-ˈde-nə-ˈsefələs
Ancylostoma	an-see-LOS-tow-mah	an-sə-ˈläs-tə-mə
Angiostrongylus	AN-jee-o-STRON-jah-lus	ˈan-jē-ə-ˈsträn-jə-ləs
Anisakis	an-ee-SAY-kiss	a-nə-ˈsā-kəs
Antoni	an-TOON-nee	an-ˈtō-nē
Ascaris	ASS-ka-ris	ˈas-kə-rəs
Babesia	bah-BEE-zh-ah	bə-ˈbē-zh-ə
Balamuthia	BAL-a-moo-thee-ah	
Balantidium	BAL-an-TID-ee-um	bal-ən-ˈtid-ē-əm
bancrofti	ban-KROFT-ee	ban-ˌkrȯft-ī
Baylisascaris	BAY-lis-as-KA-RIS	
Bignami	BEN-yah-meh	
Blastocystis	BLAS-tow-sis-TIS	
brucei	BREW-see	brü'sē-ī
cantonensis	KAN-ton-en-SIS	
ceylanicum	SCI-la-nee-cum	
Chagas	SHAh-gas	chägəs
Chrysops	KRIS-ops	kris-ˌäps
Clonorchis	KLO-nor-kis	klō-ˈnȯr-kəs
coli	KOHL-eye	kō-ˌlī
costaricensis	KOS-tar-EE-sen-sis	
Cryptosporidium	krip-tow-SPOR-i-dee-um	krip-tō-spȯr-ˈi-dē-əm
Cyclospora	SCI-KLO-spor-ah	sī-klō-ˈspȯr-ə
dendriticum	DEN-drit-IK-um	den-ˈdri-tik-əm
Dientamoeba	DYE-ant-ah-mee-bah	dī-ˌent-ə-ˈmē-bə
Diphyllobothrium	DYE-fil-ow-both-REE-um	dīˌfil-ə-bäth-rē-əm
Dipylidium	DYE-pie-LID-ee-um	dī-ˌpī-ˈlid-ē-əm
dirofilaria	DYE-row-fi-LAR-ee-a	dī-rō-fə-ˈlar-ē-ə
Dracunculus	dra-KUNG-kyoo-lus	drə-ˈkəŋ-kyə-ləs
Echinococcus	eh-KYE-no-KOK-us	i-ˌkī-nə-ˈkä-kəs
Echinostoma	eh-KYE-no-STO-mah	i-ˌkī-nə-ˈstō-mə
Endolimax	en-DOW-lie-MAKS	ˈen-də-ˈlī-ˌmaks
Entamoeba	ent-a-MEE-ba	en-tə-ˈmē-bə

Name	Pronunciation Guide	American IPA
Enterobius	EN-ter-owe-BEE-us	ent-ə-ˈrō-bē-əs
Enteromonas	EN-ter-owe-MOW-nas	ent-ə-ˈrō-ˈmō-ˌnas
falciparum	fal-SIP-pah-rum	fal-ˈsi-pə-rəm
Fasciola	fah-SEE-ow-lah	fə-ˈsē-ə-lə
Fasciolopsis	FAH-see-ow-lop-sis	ˈfă-shōˈläp-səs
gambiense	gam-BEE-in-ss	gam-bē-en'sē
Giardia	jee-ARE-dee-ah	jē-är′dē-ə
Gnathostoma	nah-THOS-tah-mah	nə-ˈthäs-tə-mə
gondii	gon-DEE	gon'dē-ī
haematobium	HEE-mah-tow-BEE-um	hē-mə-ˈtō-bē-əm
Hemiptera	heh-MIP-tah-rah	hi-ˈmip-tə-rə
Heterophyes	HET-er-OW-fye-eez	ˈhe-tə-ˌrōˈfī-ēz
histolytica	HIS-tow-lit-i-ka	his′təlit′ikə
Hymenolepis	HI-men-OL-oh-pis	hī-mə-ˈnäl-ə-pəs
kellicotti	KEL-lee-KOT-tee	
lamblia	lam-BLEE-ah	lăm′blē-ə
lanceolatum	LAN-see-o-LATE-um	ˈlan-sē-ə-ˌlāt-əm
Laveran	LAH-ver-RAH	la-və-ˈrä^{n}
Leeuwenhoek	LAY-vun-HOOK	lā-vən-ˌhu̇k
Leishmania	LEESH-ma-NEE-ah	lēsh-ˈma-nē-ə
Loa	low-ah	lo′ə
lumbricoides	lum-BRI-koy-dz	ˈləm-bri-kȯi-dz
malariae	ma-ler-EE-ay	məˈlerēˌē
malayi	MAH-la-i	
Mansonella	MAN-so-nel-ah	man-sə-ˈnel-ə
mansoni	MAN-sow-nigh	man-sə-ˌnī
medinensis	MEH-dee-NEN-sis	
Mesocestoides	MEH-zo-SES-toy-DEEZ	me-zə-ses-ˈtȯi-dēz
moshkovskii	mosh-KOV-ski-ee	
Naegleria	NAY-glir-EE-ah	nā-ˈglir-ē-ə
Nanophyetus	NAH-no-FIE-ee-tus	nānəˌfīˈētəs
Necator	ne-KAY-tor	nə-ˈkāt-ər
Oesophagostomum	IS-of-ah-GOS-tow-mum	i-ˌsäf-ə-ˈgäs-tə-məm
Onchocerca	ONG-ko-ser-kah	äŋ-kə-ˈsər-kə

Name	Pronunciation Guide	American IPA
Opisthorchis	AH-pis-thor-kis	äp-əs-ˈthȯr-kəs
ovale	OO-va-leh	ō-ˈva-lē
ozzardi	OZ-zar-DEE	
Paragonimus	par-ah-GON-i-mus	par-ə-ˈgän-ə-məs
parvum	PAAR-vum	pahr'vŭm
Plasmodium	plaz-MODE-ee-um	plaz-ˈmō-dē-əm
procyonis	pro-SCI-on-is	
rhodesiense	row-DEE-zan-ss	rō-dē-zē-en'sē
Schistosoma	SHIS-tih-sow-mah	shis-tə-ˈsō-mə
Simulium	seh-MYOO-LEE-um	si-ˈmyü-lē-əm
sinensis	SCI-nen-sis	
spinigerum	SPI-ni-G'E-rum	
stenocephala	ste-NO-seh-FAAL-ah	ste-nō-sə̇ˈfāl-ə
streptocerca	STREP-tow-ser-ka	strep-tə-ˈsər-kə
Strongyloides	STRON-ji-LOI-deez	strän-jə-ˈlȯi-ˌdēz
suis	SW-ee	swʸē
suum	SO-om	sȯ-u̇m
Taenia	TEE-nee-ah	tē-nē-ə
Ternidens	ter-nah-DENZ	tər-nə-ˌdenz
Terranova	TEAR-ah-now-vah	ter-ə-ˈnō-və
Toxocara	TOK-so-ka-rah	täk-sə-ˈkar-ə
Toxoplasma	TOK-so-plaz-ma	täk-sə-ˈplaz-mə
Trichinella	TRIK-in-el-ah	trik-ə-ˈnel-ə
trichiura	trick-ee-UR-ah	
Trichomonas	trick-oh-MOAN-us	tri-kə-ˈmō-nəs
Trichuris	TRICK-you-ris	trik-ˈyu̇r-əs
Trypanosoma	tri-PAN-oh-so-mah	tri-ˌpan-ə-ˈsō-mə
Uncinaria	un-sin-NAR-EE-ah	ən-sə-ˈnar-ē-ə
vaginalis	vaj-gi-NAL-is	vaj-ə-ˈnā-ləs
Virchow	FIR-kow	fir-kō
vivax	VYE-vax	vī-ˌvaks
volvulus	VOL-view-lus	väl-vyə-ləs
Welch	WELL-ch	welch
Wuchereria	VOOK-ah-rer-EE-ah	vu̇k-ə-ˈrir-ē-ə

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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.

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Acronyms and common terms

Introduction

Many acronyms and standard terms have been applied throughout the text. To assist the reader each acronym is defined at its first use within the chapter. As a new field matures what was once an acronym may cross over to become a standard term e.g., deoxyribonucleic acid is more commonly referred to by its acronym, DNA to the extent that it is used as if it were a word.

To aid readers new to the field, who may be unfamiliar with the standard terms, the entire list of acronyms and terms applied are available below.

Term	Meaning
AIDS	Acquired ImmunoDeficiency Syndrome
APOC	African Programme for Onchocerciasis Control
ATP	Adenosine Tri Phosphate
ATPase	Enzyme that catalyzes the decomposition of ATP (adenosine triphosphate) into ADP (adenosine di phosphate)
B cells	Bone marrow lymphocyte
B12	Vitamin B12 (cobalamin)
BCE	Before the Current Era
BID	*Bis In Die*: twice a day
Bti	*Bacillus thuringiensis israelensis*
CD36	Member of the CD36 (cluster of definition 36) proteins
$CD4^+$	Cytotoxic T helper cell (4+)
CD40	Member of the CD40 (cluster of definition 40) proteins
CD68	Member of the CD68 (cluster of definition 68) proteins
$CD8^+$	Cytotoxic T helper cell (8+)
CDC	Centers for Disease Control (United States)
Cdc42	Cell division control protein 42
cDNAs	Complementary DNA
CE	Cystic Echinococcosis
CL	Cutaneous leishmaniasis
CLM	Cutaneous larva migrans
CNS	Central Nervous System
CPK	Creatine PhosphoKinase
CSA	Chondroitin Sulfate A
CSF	CerebroSpinal Fluid
CT	Computed Tomography scan
DALYs	Deaths and Disability Adjusted Life Years

Term	Meaning
DCL	Diffuse Cutaneous Leishmaniasis
DDT	DichloroDiphenylTrichloroethane
DEC	DiEthylcarbamazine Citrate
DEET	DiEthylToluamide (N,N-Diethyl-meta-toluamide)
DFA	Direct Fluorescent Antibody test
DFID	Department for International Development
DNA	DeoxyriboNucleic Acid
D. Sc.	Doctor of Science
ELISA	Enzyme-Linked ImmunoSorbent Assay
EM	Electron Micrograph
EPT	Expedited Partner Therapy
ER	Endoplasmic Reticulum
ERCP	Endoscopic Retrograde CholangioPancreatography
ESPs	Excretory/Secretory Products
FDA	Food and Drug Administration (United States)
FDG-PET	FluoroDeoxyGlucose-Positron Emission Tomography
FGS	Female Genital Schistosomiasis
g	Gram
G6PD	Glucose-6-Phosphate Dehydrogenase
GAE	Granulomatous Amoebic Encephalitis
g/DL	Gram per deciliter
GEMS	Global Enteric Multicenter Study
GI	GastroIntestinal
GPIIb/IIIa	Integrin complex GPIIb/IIIa
GTPase	Family of hydrolase enzymes that can bind and hydrolyze guanosine triphosphate (GTP)
HAART	Highly Active Antiretroviral Therapy
HAT	Human African Trypanosomiasis
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
HIV/AIDS	Human Immunodeficiency Virus /Acquired Immunodeficiency Syndrome
HIV-1	Human Immunodeficiency Virus type 1
HLA-DR	Human Leukocyte Antigen – DR isotype (MHC class II cell surface receptor encoded by the human leukocyte antigen complex)
hr/s	Hour/s
ICAM1	InterCellular Adhesion Molecule 1
IFA	Indirect Fluorescent Antibody test

Term	Meaning
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IGRA	Interferon Gamma Release Assay
IHA	Indirect Hemagglutination Assay
IM	IntraMuscular
IV	IntraVenous
kg	Kilogram
L1	Rhabditiform larval stage
L1–L4	Larval stages 1 through 4
L2	Second-stage rhabditiform
L3	Filariform
LAMP	Loop Mediated isothermal amPlification
LF	Lymphatic Filariasis
mcg	One millionth (1/1,000,000) gram
MCL	MucoCutaneous Leishmaniasis (also known as espundia)
M.D.	*Medicinae doctor*: doctor of medicine
MENA	Middle East and North Africa
mf/ml	Microfilaria per ml
mg	Milligram
mg/dL	Milligram per deciliter
mmol/L	Milli mol per liter
ml	Milliliter
MRI	Magnetic Resonance Imaging
mRNA	messenger RNA
NAAT	Nucleic Acid Amplification Testing
NO	Nitric Oxide
OCP	Onchocerciasis Control Program
OEPA	Onchocerciasis Elimination Program for the Americas
OLM	Ocular Larva Migrans
O+P	Ova & Parasite examination
PAIR	Puncture, Aspiration, Injection, Re-aspiration
PAM	Primary Amoebic Meningoencephalitis
PCR	Polymerase Chain Reaction
PfEMP1	*Plasmodium falciparum* Erythrocyte Membrane Protein 1
PfHRP2	*Plasmodium falciparum* Histidine-Rich Protein-2

Term	Meaning
pH	Scale used to specify the acidity or basicity of an aqueous solution
Ph.D	*Doctor philosophiae*: doctor of philosophy
PKDL	Post-Kala-azar Dermal Leishmaniasis
PO	*Per Os*: by mouth
PPD	Purified Protein Derivative
PVA	PolyVinyl Alcohol
qnhrs	*Quaque "n" hora*: every 'n' hours
Q-day	*Duaque Die*: every day i.e., 1 per day
QID	*Quater In Die*: 4 times a day
RNA	RiboNucleic Acid
rRNA	Ribosomal RNA
rpm	Revolutions Per Minute
SARS	Severe Acute Respiratory Syndrome
sp	Species (singular)
spp	Species (plural)
STARI	Southern Tick-Associated Rash Illness
STHs	Soil-Transmitted Helminths
Sr	Senior
T cells	Thymus leukocyte
T helper cell	Thymus lymphocyte helper cell
Th1	Type 1 T helper cell
Th2	Type 2 T helper cell
TID	*Ter In Die*: three times per day
TNF	Tumor Necrosis Factor
TPE	Tropical Pulmonary Eosinophilia
U.S.	United States
USAID	United States Agency for International Development
UV	Ultra Violet light
VL	Visceral Leishmaniasis
VSG	Variant Surface Glycoprotein

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