

**CDC 4T0510**

**Medical Laboratory  
Journeyman-Microbiology**

**Volume 4. Parasitology**



**Air Force Institute for Advanced Distributed Learning**

**The Air University**

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CLINICAL PARASITOLOGY has kept abreast with the enormous changes in medical and public health science. This, the final volume, presents information on parasites of medical importance, including helminths and protozoa. Using full-page life-cycle illustrations, we hope that you will obtain a clearer understanding of the parasites covered and their relationship to the environment and, particularly, to humans. Emphasis has also been placed on illustrating the stage of the parasite of greatest importance in the laboratory diagnosis. Of the five units found here, Unit 1 addresses the taxonomy, definitions, specimen collection, and diagnostic procedures. Unit 2 explores the lumen-dwelling helminths including nematodes, cestodes, and trematodes of clinical importance. Unit 3 is a brief look at tissue and blood helminths. Unit 4 provides an overview of the lumen-dwelling protozoa, and Unit 5 concludes with the blood and tissue protozoa.

Foldouts 1 through 3 are bound in the rear of this volume. Use them as the text directs.

A glossary of terms used in this course is included at the end of this volume.

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This volume is valued at 24 hours and 8 points.

## Acknowledgments

PREPARATION of this volume was greatly aided by the use of the Centers for Disease control parasite life cycle charts found at <http://www.dpd.cdc.gov/dpdx/>. All parasite life cycle charts used in this volume came from this source.

### NOTE:

In this volume, the subject matter is divided into self-contained units. A unit menu begins each unit, identifying the lesson headings and numbers. After reading the unit menu page and unit introduction, study the section, answer the self-test questions, and compare your answers with those given at the end of the unit. Then do the unit review exercises.

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## **Student Notes**

**Please read the unit menu for Unit 1 and begin ➡**

# Unit 1. Laboratory Methods in Medical Parasites

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**B**ECAUSE most parasites are very small in size, they were not detected until Antony van Leeuwenhoek developed the microscope. He wrote about his discoveries to the Royal Society of London in a series of letters covering a period between 1674 and 1716. It seems he found *Giardia lamblia* in his own diarrheic stools, as the following quote reveals.

*“My excrement being so thin, I was at divers times persuaded to examine it; and each time I kept in mind what food I had eaten, and what drink I had drunk, and what I found afterwards. I have sometimes seen aminmalules a-moving very prettily....”*

A. van Leeuwenhoek on November 4, 1681

As you can see, parasitic protozoa were being reported as early as the seventeenth century. Today, there are at least 45,000 described species of protozoa and helminths. Many of these organisms are parasitic. As you recall from your technical training, a parasite is a plant or animal that lives upon or within another living organism. It draws its nourishment and/or obtains some advantage at the expense of the other living organism. Hence, parasitology is the science or study of these parasites and their relationship with their host. Accurate clinical diagnosis of parasitic diseases is difficult, and laboratory confirmation is usually necessary. Demonstrating the diagnostic stage or stages of the parasite by direct examination of the specimen is the most reliable method of establishing a diagnosis of most parasitic infections. In this unit, you will study parasite classification, specimen collection and processing, and diagnostic procedures to be accomplished by the laboratory.

## 1-1. Parasitology

All of us in the health care field readily accept the fact that the world around us is teeming with microorganisms. However, for most of the western world, the existence of parasites and parasitic infections somehow seems remote. Mentally, we relegate parasitology to exotic tropical areas of the world and feel it has little relevance to our work in Air Force laboratories. Some of us may even feel that, when the need to know about parasites arises, such as when we receive orders to a remote part of the world, we can “bone up” on the subject to meet the demands of our new assignment. But, ask yourself, “Is this a realistic view of the relevance of parasitology to your job?”

Several factors in today’s world support a negative response to this question. As a nation, we and the world in general, have become increasingly mobile. This underlines a trend favoring the transportation and establishment of parasitic reservoirs beyond the boundaries of their traditional habitats. For another, increasingly refugee populations are coming to the United States from endemic areas, and may unknowingly harbor parasites. Also, the prevalence of immunocompromised patients must be considered since they are at high risk for parasitic infections. Therefore, it is important that laboratorians be trained in recovery and identification procedures for parasites.

Parasitology from an exclusively military viewpoint should focus on the areas of the world where members are often deployed. Many of these areas are notorious hotbeds for parasitic diseases. The debilitating nature of parasitic action can affect how the United States conducts battle operations. You

should always be aware that any parasitic infection can have a drastic effect on military operations and an example of this will be illustrated in our discussion of leishmaniasis.

### 601. Parasite classification and definitions 17.6.3C

Only a few people realize that there are far more kinds of parasitic than nonparasitic organisms in the world. Organisms that are not parasites are usually hosts; some parasites are even hosts. If the viruses rickettsias and parasitic bacteria and fungi were excluded, the parasites would still be the majority. “How does one begin to study or identify such an elaborate, diverse group of microorganisms?” The answer is through the science of classification-*taxonomy*.

#### Taxonomy

You may be saying to yourself “Oh no! Not taxonomy again.” It may not seem important, but without it, the identification of all living organisms is chaotic. Just like the bacteria and fungi, all parasites are classified into a kingdom, phylum, class, order, family, genus, and species. Parasites are in the Protista Kingdom. They are divided into the following subkingdoms based on cellular characteristics:

- Subkingdom *Protozoa* (unicellular organisms).
- Subkingdom *Metazoa* (multicellular organisms).

The unicellular organisms produce protozoan infections and the multicellular organisms produce helminthic infections in humans.

#### Evolution of parasite taxonomy

In 1758, the formal science of taxonomy was started with the tenth edition of *Systema Naturae*, which was published by Linnaeus. Linnaeus is credited with the description of the sheep liver fluke *Fasciola hepatica*. The early progenitors of this system divided organisms based on their macroscopic or microscopic descriptions. Today, they rely on these published species descriptions, as well as on studies of DNA, proteins, ecological function, and geographical distribution to develop phylogenies of parasites.

#### Taxonomy of parasites

The table below is a brief overview of the current taxonomy of the parasites.

| Taxonomic Designation                          | Most Common Human Parasite Genera                                                                                                                                             |
|------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Subkingdom: <i>Protozoa</i></b>             |                                                                                                                                                                               |
| —Phylum: <i>Sarcomastigophora</i>              |                                                                                                                                                                               |
| ——Subphylum: <i>Sarcodina</i> (amebae)         | <i>Entamoeba</i> , <i>Iodamoeba</i> , <i>Endolimax</i> , <i>Naegleria</i> , <i>Acanthamoeba</i> , <i>Balamuthia</i> , and <i>Blastocystis</i> species                         |
| ——Subphylum: <i>Mastigophora</i> (flagellates) | <i>Giardia</i> , <i>Dientamoeba</i> , <i>Chilomastix</i> , <i>Retortamonas</i> , <i>Enteromonas</i> , <i>Trichomonas</i> , <i>Leishmania</i> , and <i>Trypanosoma</i> species |
| —Phylum: <i>Ciliophora</i> (ciliates)          | <i>Balantidium coli</i>                                                                                                                                                       |
| —Phylum: <i>Apicomplexa</i> (apicomplexans)    |                                                                                                                                                                               |
| ——Class: <i>Sporozoea</i>                      |                                                                                                                                                                               |
| ————Subclass: <i>Piroplasma</i>                | <i>Babesia</i> species                                                                                                                                                        |
| ————Subclass: <i>Coccidia</i>                  | <i>Plasmodium</i> , <i>Cryptosporidium</i> , <i>Cyclospora</i> , <i>Isospora</i> , and <i>Toxoplasma</i> species                                                              |



| <b>Taxonomic Designation</b>                | <b>Most Common Human Parasite Genera</b>                                                                                                                                                                                                                                                       |
|---------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| —Phylum: <i>Microspora</i> (microsporidia)  | <i>Encephalitozoon</i> , <i>Enterocytozoon</i> , and <i>Septata</i> species                                                                                                                                                                                                                    |
| <b>Subkingdom: <i>Metazoa</i></b>           |                                                                                                                                                                                                                                                                                                |
| —Phylum: <i>Platyhelminthes</i> (flatworms) |                                                                                                                                                                                                                                                                                                |
| —Class: <i>Cestoidea</i> (tapeworms)        | <i>Diphyllobothrium</i> , <i>Dipylidium</i> , <i>Echinococcus</i> , <i>Hymenolepis</i> , and <i>Taenia</i> species                                                                                                                                                                             |
| —Class: <i>Trematoda</i> (flukes)           | <i>Clonorchis</i> , <i>Fasciola</i> , <i>Fasciolopsis</i> , <i>Heterophyes</i> , <i>Metagonimus</i> , <i>Nanophyetus</i> , <i>Opisthorchis</i> , <i>Paragonimus</i> , and <i>Schistosoma</i> species                                                                                           |
| —Phylum: <i>Nematoda</i> (roundworms)       |                                                                                                                                                                                                                                                                                                |
| —Class: <i>Adenophorea</i> (Aphasmidia)     | <i>Trichinella</i> , <i>Trichuris</i> , and <i>Capillaria</i> species                                                                                                                                                                                                                          |
| —Class: <i>Secernentia</i> (Phasmidia)      | <i>Enterobius</i> , <i>Ascaris</i> , <i>Ancylostoma</i> , <i>Necator</i> , <i>Strongyloides</i> , <i>Trichostrongylus</i> , <i>Anisakis</i> , <i>Wuchereria</i> , <i>Brugia</i> , <i>Loa</i> , <i>Onchocerca</i> , <i>Mansonella</i> , <i>Dracunculus</i> , and <i>Angiostrongylus</i> species |

### Definitions

Before we go further into our study, we should review some basic definitions and terms associated with parasitology.

#### **Host**

A host is an organism in or on which a parasite lives.

#### **Intermediate host**

The intermediate host is required in the life cycle of the parasite for larval development. This development must occur before the parasite is infectious to the definitive host or the secondary intermediate host.

#### **Definitive host**

The definitive host is where the sexual reproduction of a parasite takes place.

#### **Vector**

A vector is a carrier (arthropod or animal) that transfers an infective agent from one host to another.

#### **Obligate parasite**

An obligate parasite is a parasite that must always live in contact with the host.

#### **Ectoparasite**

An ectoparasite is an organism that lives on or within the skin of its host; for example lice, mites, and ticks.

#### **Endoparasite**

An endoparasite is an organism that lives within the body of the host.

#### **Commensalism**

A commensalism is an association in which one organism receives benefits but the other organism is neither helped nor harmed.

***Mutualism***

A mutualism is an association in which both organisms benefit and cannot survive without each other.

***Symbiosis***

A symbiosis is close association between two different organisms (living together).

***Hermaphroditism***

Hermaphroditism is the presence of both male and female reproductive systems in the same individual organism.

***Lumen***

A lumen is the cavity of a hollow, tubular organ, such as the intestines or blood vessels.

***Cyst***

A cyst is a part of the life cycle of the protozoa (amebae, flagellates, or ciliates) during which they are enclosed within a protective wall and are dormant.

***Trophozoites***

Trophozoites are the active, motile, feeding stage of protozoa (amebae, flagellates, or ciliates), as contrasted with the nonmotile encysted stage.

***Eggs***

An egg is the female reproductive cell of the helminths (worms) before fertilization, after fertilization, or even after some development.

***Larva***

Larva is the dependent, motile, sometimes feeding, developmental stage in the life cycle of a helminth (worm). Rhabditiform larvae are rod-shaped and usually noninfective. Filariform larvae resemble threadlike filariae and are usually infective.

***Organelles***

Organelles are the membrane-bound cytoplasmic structures with a distinctive morphology and function. They are present in all eukaryotic cells. Examples include the nucleus, mitochondria, lysosomes, peroxisomes, Golgi apparatus, and endoplasmic reticulum, as well as chloroplasts in plants and cilia, flagella, and the cytopharynx in protozoa.

***Epidemic***

Epidemic is a disease that spreads rapidly and infects many people in a community or area usually within a short time frame.

***Endemic***

Endemic is a population or geographical area where a disease or agent is present or usually prevalent at all times.

***Life cycle of parasites***

The life cycle of parasites that are important to humans vary in complexity. They range from a simple pattern of person-to-person contact with no intermediate host, to the need for one intermediate host, or, to the involved pattern of those that require two intermediate hosts. Life cycles are discussed in detail for each of the parasites studied in subsequent units.

TYPICAL INFECTION SITES OF SELECTED PARASITES

| S I T E                | TISSUE:<br>BLOOD [B]<br>LYMPH [L]<br>MUSCLE [M]<br>SUBCUTANEOUS [SC] | TYPICAL<br>LOCATION(S)<br>FOUND | DIGESTIVE<br>TRACT/<br>INTESTINES | LIVER &<br>DUCTS | BLADDER          | LUNG            | BRAIN           | HUMAN AS<br>INCIDENTAL/<br>ACCIDENTAL<br>HOST |
|------------------------|----------------------------------------------------------------------|---------------------------------|-----------------------------------|------------------|------------------|-----------------|-----------------|-----------------------------------------------|
| <b>O R G A N I S M</b> |                                                                      |                                 |                                   |                  |                  |                 |                 |                                               |
|                        | <b>Filaria</b>                                                       |                                 | <b>Hookworm</b>                   |                  |                  |                 |                 |                                               |
|                        | B. malayi                                                            | B, L                            | A. duodenale                      | E. histolytica*  | S. japonicum     | P. westermani ★ | N. fowleri      | D. immitis                                    |
|                        | L. loa                                                               | SC                              | N. americanus                     | F. hepatica      | S. haematobium   | E. histolytica* | E. histolytica* | E. granulosis                                 |
|                        | M. ozzardi                                                           | B                               | A. lumbricoides                   |                  | T. vaginalis     | P. jiroveci     |                 | T. canus                                      |
|                        | M. perstans                                                          | B                               | B. coli                           |                  | E. vermicularis* |                 |                 | D. canium                                     |
|                        | M. streptocerca                                                      | SC                              | C. meslinii                       |                  |                  |                 |                 |                                               |
|                        | O. volvulus                                                          | SC                              | D. fragilis                       |                  |                  |                 |                 |                                               |
|                        | W. bancrofti                                                         | B, L                            | D. latum                          |                  |                  |                 |                 |                                               |
|                        | D. medinensis                                                        | SC                              | E. histolytica ♦                  |                  |                  |                 |                 |                                               |
|                        | L. eishmania spp                                                     | SC                              | E. vermicularis ♦                 |                  |                  |                 |                 |                                               |
|                        | T. spiralis                                                          | M                               | E. coli                           |                  |                  |                 |                 |                                               |
|                        | Trypanasoma spp.                                                     | B                               | H. diminutia                      |                  |                  |                 |                 |                                               |
|                        | Babesia spp.                                                         | B                               | H. nana                           |                  |                  |                 |                 |                                               |
|                        | Plasmodium spp.                                                      | B                               | I. butschlii                      |                  |                  |                 |                 |                                               |
|                        |                                                                      |                                 | I. belli                          |                  |                  |                 |                 |                                               |
|                        |                                                                      |                                 | S. japonicum                      |                  |                  |                 |                 |                                               |
|                        |                                                                      |                                 | S. mansoni                        |                  |                  |                 |                 |                                               |
|                        |                                                                      |                                 | T. solium                         |                  |                  |                 |                 |                                               |
|                        |                                                                      |                                 | T. saginata                       |                  |                  |                 |                 |                                               |
|                        |                                                                      |                                 | S. stercoralis                    |                  |                  |                 |                 |                                               |
|                        |                                                                      |                                 | T. trichuria                      |                  |                  |                 |                 |                                               |
|                        |                                                                      |                                 | G. intestinalis<br>(lamblia)      |                  |                  |                 |                 |                                               |
|                        |                                                                      |                                 | Cryptosporidium                   |                  |                  |                 |                 |                                               |
|                        |                                                                      |                                 | F. buski                          |                  |                  |                 |                 |                                               |

♦=Primary infection site \* = Important secondary infection sites. ★ =Primary site, but may be widely dispersed.

## **602. Fecal specimen collection and preservation**

The ability to successfully detect and identify parasites depends on proper specimen collection, preservation, and processing. The most common anatomical site for parasitic infection is the gastrointestinal tract; therefore, the most commonly submitted diagnostic specimen is feces. However, other specimens include vaginal and urethral discharges, prostatic secretions, urine, sputum, aspirates, blood, and biopsy material.

### **Safety**

As always, treat all parasitology specimens as if they were potentially infectious. Follow all safety precautions including proper labeling of hazardous preservatives and reagents; use of biological safety cabinets and proper centrifugation containers; and follow acceptable biohazard disposal policies and Universal Precautions guidelines.

### **Specimen collection**

As with the study of other microorganisms in the laboratory, appropriate specimens must be collected from the patient. Also, they must be transported to the laboratory in a condition preserved sufficiently to allow the detection and identification of any parasitic forms that may be present. Special guidelines and considerations are recommended to guarantee proper collection for accurate examination of fecal specimens.

### ***General considerations***

Interfering substances that obscure or cause a decline in the number of protozoan organisms passed include radiologic reagents, such as barium sulfate; certain medications; mineral oil, bismuth, nonabsorbable antidiarrheal preparations, and antimalarials; and some antibiotics (for example, tetracyclines). Because of the excess crystalline material in the stool specimen, intestinal protozoa may be impossible to detect for 5 to 10 days after the use of barium. Protozoan organisms may be difficult to detect for several weeks after the administration of medication is halted. Contamination of the specimen with water, urine, soil, etc., should be avoided since protozoan organisms may lose their motility or undergo lyses due to such contamination. Urine, also, destroys trophozoites and may cause helminth eggs to hatch. Such contamination may also be a source of “free-living” protozoans that can be mistaken for parasites.

### ***Specimen container***

Stool specimens should be collected directly during bowel movement into a clean, wide-mouthed container, with a tight-fitting lid. Fit the lid tightly to the container immediately after collecting the sample in order to maintain adequate moisture. Each specimen container must be labeled properly. For safety purposes, specimen containers must be placed in a zip-lock (plastic) bag for transport to the laboratory. It is helpful to have information on relevant travel history and/or presumptive diagnosis.

### ***Number of specimens***

Numerous factors determine the number of specimens required for submission to demonstrate intestinal parasites. These include:

- Specimen quality.
- Method accuracy.
- Infection severity/parasite burden.

Usually three specimens collected prior to treatment is recommended, with two collections being normal bowel movements and the third to follow a cathartic (never oil based) substance. Never use laxatives if the patient has abdominal pain or diarrhea. Examining six specimens will isolate 90 percent of parasitic infections. To detect amebiasis, the collection of at least six specimens is recommended. A series of three specimens should be collected in no more than 10 days and a series of six fecal collections should be collected in no more than 14 days. Collection on alternate days is

recommended because detectable levels of the organism's existence are not always prevalent each day. Following treatment, the patient should submit more fecal specimens for examination. Typically, a series of three is recommended but this can vary based on the diagnosis and other factors. Some general guidelines for post therapy follow-up based on the type of parasitic infection are as follows:

| Parasitic Infection | Test for Cure Following Treatment |
|---------------------|-----------------------------------|
| Protozoan           | 3–4 weeks                         |
| Helminth            | 1–2 weeks                         |
| Taenia              | 5–6 weeks                         |

### ***Time of specimen examination***

Fresh specimens are mandatory for the recovery of motile trophozoites. Since the age of the specimen directly influences the recovery of protozoan organisms, record the collection time on the laboratory request. Liquid specimens should be examined within ½ hour after *passage* (not 30 minutes from the time they reach the laboratory), or the specimen should be placed in polyvinyl alcohol (PVA) fixative or another suitable preservative. Examine semi-formed or soft specimens within 1 hour of passage; if this is not possible, preserve the stool material. Although the time limits are not as critical for the examination of a formed specimen, it is recommended that the material be examined on the day of passage. If these time limits cannot be met, preserve portions of the sample. Do not hold stool specimens at room temperature for more than 3 hours; refrigerate them at 3 to 5°C in closed containers to prevent desiccation. At this temperature, eggs, larvae, and protozoan cysts remain viable for several days. Never incubate or freeze fecal specimens prior to examination. When proper criteria for collection of fecal specimens are not met, the laboratory should request additional samples.

### **Specimen preservation**

If specimens cannot be examined immediately upon arrival to the laboratory, because of the time of arrival (for example, nights and weekends), workload, and availability of trained personnel, they must be preserved. Appropriate preservation will maintain protozoan morphology and prevent further development of certain helminth eggs and larvae. Some acceptable preservation methods are listed below:

1. Maintain at room temperature and examine within 3 hours.
2. Maintain at refrigerator temperatures for not more than three days before completing examination.
3. Place specimen in a preservative solution if examination cannot be completed within timeframes listed in 1 or 2 above, or if the examination will be delayed for an unknown amount of time.

Remember, method one 1 above does not apply to liquid stools, which should be examined within ½ hour after passage. In this text, we concentrate our discussion on method 3—using preservative solutions.

### **Four preservative solutions**

The following four preservative solutions are reviewed:

1. Formalin.
2. Polyvinyl alcohol (PVA).
3. Merthiolate-iodine-formalin (MIF).
4. sodium acetate-acetic acid-formalin (SAF).

When selecting an appropriate fixative, it is important to realize its limitations. Ideally, the solution preserves all diagnostic stages of all possible intestinal parasites, while being completely compatible with the diagnostic procedures required to positively identify them. Unfortunately, this is not the case with many preservative solutions. MIF, SAF, and PVA solutions do preserve fecal samples

adequately and are compatible with the two most common diagnostic procedures (preparation of permanently stained smears and concentrating the specimen). Permanently stained smears greatly increase accuracy in the identification of protozoa and concentration procedures, and they enhance your chances of detecting all intestinal parasitic organisms.

**NOTE:** Regulatory requirements related to waste disposal impact the use of preservatives with mercury compounds. Copper sulfate or a zinc based compound usually replaces the mercuric compound in the Schaudinn's fixative. However, the zinc compound provides better results than the copper sulfate, but neither of the compounds work as well as the mercuric compound for the preservation of protozoan morphology.

### ***Formalin***

Protozoan cysts, helminth eggs, and larvae are well preserved for long periods in 5 or 10 percent formalin, which has been used for years as an all-purpose fixative. To help maintain morphology, the formalin can be buffered with sodium phosphate buffers. Usually, 5 percent formalin is used for preserving protozoan cysts and 10 percent formalin is for helminth eggs and larvae. It is recommended that hot formalin (60°C) be used for helminth eggs to prevent further development of the eggs to the infective stage, especially when using 5 percent formalin. Formalin should be used in the ratio of three-parts formalin to one-part fecal material. It is important to thoroughly mix the fresh specimen and formalin to ensure good preservation. Remember, formalin is a preservative and can only be used for concentration procedures and wet mounts, *not* for permanently stained smears.

### ***Polyvinyl alcohol (PVA)***

Polyvinyl alcohol fixative solution is highly recommended as a means of preserving protozoan cysts and trophozoites for later examination. This use of PVA also permits specimens to be shipped to a reference laboratory by regular mail service for subsequent examination. PVA, a combination of modified Schaudinn's fixative and a plastic resin, should be used in the ratio of three parts PVA to one part fecal material. Perhaps the greatest advantage in the use of PVA is that permanently stained slides can be prepared from PVA-preserved material. This is not the case with many other preservatives. In fact, some permit the specimen to be examined as a wet preparation only—a technique that may not be adequate for the correct identification of protozoan organisms. PVA can be prepared in the laboratory or purchased commercially. This fixative remains stable for long periods (months to years) when kept in sealed containers at room temperature.

### ***Merthiolate-iodine-formalin (MIF)***

Another satisfactory preservative for stool specimens is the MIF solution (Sapero and Lawless), which preserves trophozoites as well as cysts, eggs, and larvae. Helminth eggs, cysts, and some trophozoites can be identified in wet mounts using this solution. However, the identification of trophozoites should be confirmed by permanent staining techniques. Although it is possible to prepare permanently stained smears from feces preserved with MIF, many parasitologists prefer to use a portion of the patient's sample that has been placed in an alternative fixative solution, such as PVA.

### ***Sodium acetate-acetic acid-formalin (SAF)***

This liquid fixative uses sodium acetate to buffer formalin and ensures good preservation of the specimen while stabilizing the morphology of any organisms present. The solution is prepared easily and has a long shelf life. However, one disadvantage experienced with using this type of preservative, is getting the preserved sediment to adhere to a glass slide. The use of Mayer's albumin has been suggested to overcome this problem, but the procedure may still cause inexperienced technicians more difficulty than the use of alternative preservative solutions.

### **Multi-vial collection kits**

To provide both the patient and the servicing laboratory with convenient procedures for the collection and handling of fecal specimens, tailor-made collection kits can be ordered from a commercial

supplier. The manufacturers can package the kits according to the individual needs of your laboratory. They will also include a multi-language instruction sheet. The patient receives a wide-mouth container to collect the fecal specimen and a collection kit from the laboratory. The lids on the vials have an attached “scoop” or “spoon” for transferring the specimen from the wide-mouth container into the vials. The kits may include one or more of the following vials or tubes:

### ***Clean vial***

The plain clean vial, as shown in figure 1-1, provides an unpreserved specimen for occult blood testing and enables the laboratory to observe the specimen consistency.

### ***Zn-PVA fixative vial***

A Zn-PVA fixative vial, as shown in figure 1-2, contains 15 ml of zinc sulfate and polyvinyl alcohol.

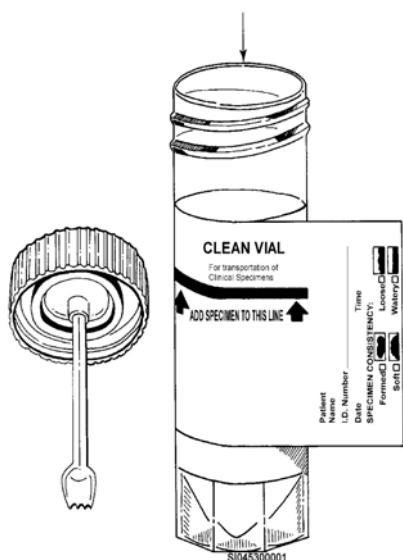


Figure 1-1. Clean vial.

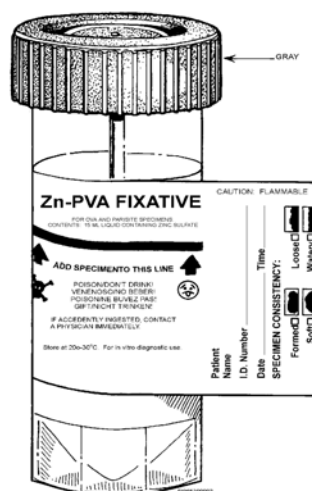


Figure 1-2. PVA fixative vial.

### ***Ten percent buffered neutral formalin vial***

A 10 percent buffered neutral formalin vial is shown in figure 1-3.

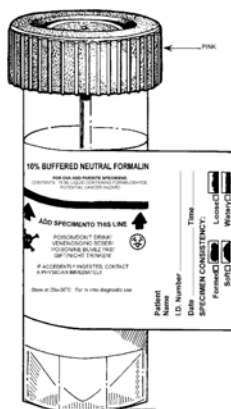


Figure 1-3. 10% Buffered formalin vial.

***Cary Blair vial***

A Cary Blair vial is shown in figure 1-4. A Cary Blair transport media with an indicator for stool cultures can be added when a request includes both culture and ova and parasite (O and P) examination.

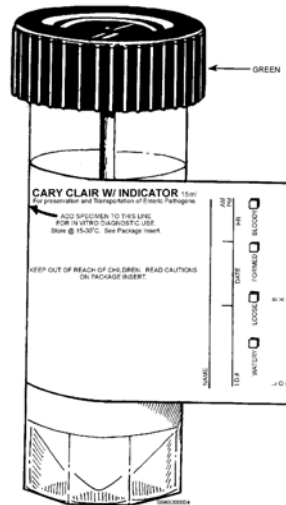


Figure 1-4. Cary Blair transport media with an indicator.

**Advantages**

The collection kits reduce the lag time between when the specimen is passed and when it is fixed. This results in better organism morphology for identification. The flexibility of the kits is another plus.

**603. Other intestinal and nonintestinal specimens**

In addition to the gastrointestinal system, some parasites infect or have an affinity for the different systems in the human body.

**Other intestinal specimens**

When repeated fecal specimen examinations fail to recover or reveal the presence of protozoa or helminths, other specimens may need to be collected. Some intestinal parasites require different collection procedures.

***Cellophane tape method for examination of pinworm***

*Enterobius vermicularis* (pinworm) is a nematode that is found worldwide and commonly encountered in children. The adult female worm migrates from the anus (usually at night) to the perianal area. She deposits her eggs on the perianal region rather than within the intestinal tract. Because of this peculiar migratory habit, the eggs are not ordinarily found in fecal specimens. Diagnosis is generally made by the recovery of the typical eggs on the cellophane tape or a commercial “pinworm paddle.” Specimens are collected first thing in the morning before the patient goes to the bathroom or bathes. Since the responsibility for collecting the specimen usually rests with the patient, or parents of the patient, explicit instructions should be provided. An illustration of the cellophane tape method, as shown in figure 1-5, can be useful. The patient should understand that several daily collections might be necessary, since migration of the female may not occur every night. The person collecting the specimen should also be instructed to wash their hands thoroughly after each collection. At least a series of four to six consecutive negative slides should be observed before the patient is considered free of the infection.



### ***Sigmoidoscopic material***

The collection of this material is usually associated with cases in which the patient is suspected of harboring *Entamoeba histolytica* or other amebae, but all stool samples are negative for parasites. However, this procedure does not take the place of routine fecal examinations; a series of at least three (six is preferable) fecal specimens should be submitted for each patient having a sigmoidoscopy examination. Ideally, the material should be collected after a spontaneous bowel movement. If the patient has been given a laxative, the sigmoidoscopy should be performed 2 to 3 hours after a bowel movement. The objective of this collection technique is to obtain material from the mucosal surface of the large intestine. The material from the mucosa is removed through aspiration or by scraping. At least six representative mucosal areas or any lesions should be aspirated or scraped. A variety of aspiration and scraping devices are available for the collection of these samples. Cotton swabs rarely provide a satisfactory sample since the organism is likely to be absorbed into the cotton. If they must be used, most of the cotton should be removed, with just enough left on the swab to safely cover the end. The remaining cotton is tightly wound around the swab to minimize the absorption of the specimen into the cotton.

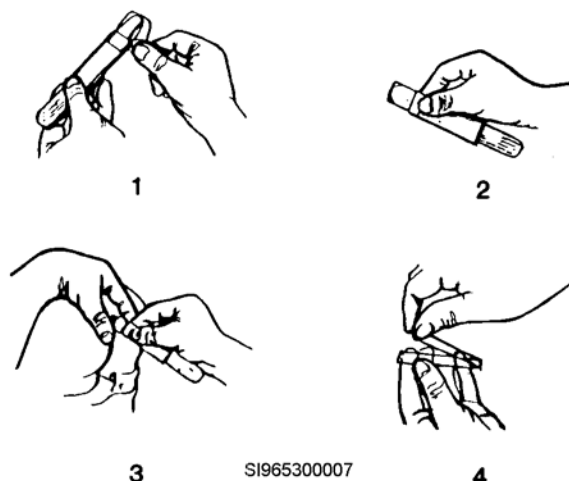


Figure 1-5. Collection of *Enterobius vermicularis* eggs using cellophane tape method.

### ***Processing material***

Once the specimen has been obtained, its handling and processing should be expedited. You should perform as many examinations as the specimen volume will allow. When there is sufficient specimen available, you should prepare wet preparations and permanent stained smears. Examine the direct wet mount immediately for the presence of moving trophozoites. Trophozoite motility may not be readily apparent, as they may need a short time to acclimate in the wet mount. Macrophages and other tissue cells can closely resemble some protozoans so any suspect findings must be confirmed from the permanent stained slide.

### ***Duodenal specimens***

In infections with *Strongyloides stercoralis* and *Giardia lamblia*, duodenal drainage often reveals organisms when stool specimens are negative, because they inhabit the duodenum or bile duct. Duodenal specimens, obtained by either intubation or the Entero-Test (string test), should be sent to the laboratory unpreserved and examined within 1 hour.

### ***Intubation samples***

If the intubation and aspiration procedure is used, the specimen should be centrifuged and the sediment examined in a direct wet mount for motile organisms. Several mounts should be prepared

and examined. Because of the dilution factor, the organism may be difficult to recover with this technique. When motile organisms are seen, a second preparation in a drop of iodine may be helpful in highlighting the characteristic internal structures and facilitating a definitive identification. If the specimen cannot be examined within 1 hour, it can be preserved using a small amount of 5 or 10% buffered formalin or SAF.

#### *Entero-Test or string test samples*

The easiest way to obtain a duodenal sample is by using the Entero-Test or string test. As illustrated in figure 1-6, the string test uses a gelatin capsule with tightly coiled, weighted, nylon yarn inside. A part of the yarn protrudes out of the capsule. This piece of yarn is taped to the side of the patient's face. The capsule is swallowed and then it dissolves in the stomach. The weighted string is carried by peristalsis into the duodenum. After 4 hours, when the string is retrieved, the weight slips off and is passed in the stool. The string is saturated with bile-stained mucus. The mucus is "scrapped" off the string into a petri dish. The sample is examined *immediately* in a direct wet mount. Whether the sample is collected by intubation or the string test, the typical "falling leaf" motility of *Giardia lamblia* is usually not visible, because the organism is trapped in the mucus. Instead, the motility will be observed as a rapid fluttering of the flagella.

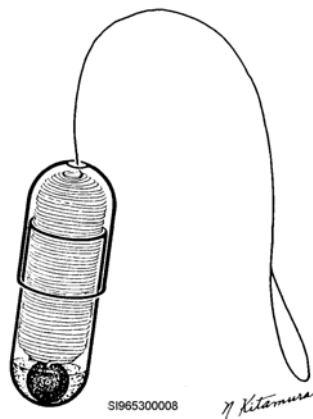


Figure 1-6. Entero-Test capsule for duodenal samples.

#### **Other nonintestinal specimens**

In this area, we turn our attention to a group of miscellaneous specimens that can harbor a variety of parasites. You must be aware of the different organisms that may be found in these specimens and be proficient at detecting and identifying them.

#### ***Sputum***

Various diagnostic stages of a number of parasites can be found in sputum specimens and may cause pneumonia, pneumonitis, or Loeffler's syndrome. Due to the life cycle of the trematode *Paragonimus westermani*, in which the adult organism lives in the lungs and passes its eggs or ova into the alveoli, this organism is probably the one most technicians associate with sputum specimens. However, the larvae of hookworms, *Ascaris lumbricoides*, and *Strongyloides stercoralis* can be recovered in the sputum if the specimen is collected during their migration through the lungs. Likewise, the trophozoites of *Entamoeba histolytica*, *Entamoeba gingivalis*, and *Trichomonas tenax*; oocysts of *Cryptosporidium parvum*; and *Pneumocystis jiroveci* (formerly *P. carinii*); can also be seen in sputum specimens. Finally, on rare occasions, the immature protoscolices or hooklets of *Echinococcus granulosus*—which resemble the cysticercoids of *Hymenolepis* organisms—can be present. Refer to figure 1-7. The scoleces contaminate the sputum when a hydatid cyst of the lung ruptures, releasing fluid containing the protoscolices, which are commonly referred to as *hydatid sand*.

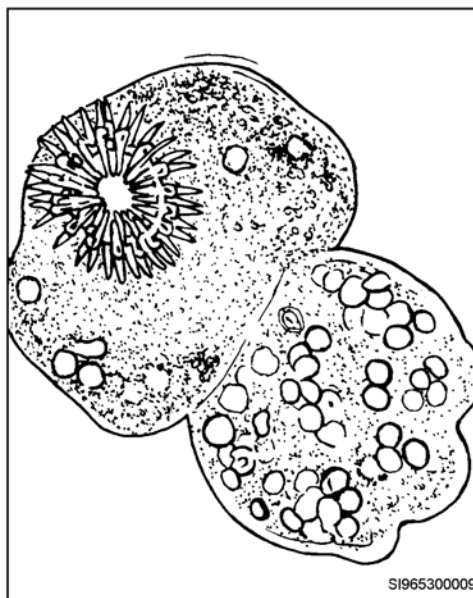


Figure 1-7. Protoscolex of *Echinococcus granulosus* removed from a hydatid cyst.

### Processing

Ideally, an early morning sputum specimen should be submitted for examination. It should be collected as soon as the patient awakens and before eating or brushing their teeth. In cases of suspected amebiasis, a direct wet mount, with or without iodine, should be performed immediately for the detection of trophozoites. If this is not practical and a delay is anticipated, the specimen should be preserved in 10 percent formalin and in PVA or SAF for later permanent staining. A very mucoid or thick sputum can be digested and concentrated by the addition of digestant and centrifugation. Equal amounts of undiluted chlorine bleach or 3 percent sodium hydroxide (NaOH) may be used for this purpose. *Paragonimus westermani* infections produce viscous sputum, streaked with blood and tinged with brownish flecks. These flecks are clusters of eggs and are described as iron filings. If *Cryptosporidium* species is suspected, then acid-fast staining should be performed. Induced sputum may be required for identification of *Pneumocystis carinii* and to differentiate trophozoite and cyst forms from other possible causes of pneumonia, particularly in the AIDS patient.

**NOTE:** Be careful not to confuse *Entamoeba gingivalis*, which may be found in the mouth, with *Entamoeba histolytica* from a pulmonary abscess. *E. gingivalis* will contain ingested polymorphonuclear neutrophils (PMN); whereas *E. histolytica* will not contain these.

### Urine and urogenital specimens

The two most commonly encountered parasitic organisms found in urogenital specimens are *Trichomonas vaginalis* and *Schistosoma haematobium*. Of these, *T. vaginalis* is by far much more common in the United States.

#### Urine specimens

Urine specimens are used in the diagnosis of *Trichomonas vaginalis* and *Schistosoma haematobium* infections. The optimal urine specimen for revealing eggs of *S. haematobium* is the last portion of a midday specimen collected after 10 to 15 minutes of strenuous exercise, such as running in place. To increase the chances of detecting any parasitic infection that may be present, it is suggested that specimens be collected on three consecutive days, since the passage of *S. haematobium* eggs fluctuates.

### *Urogenital specimens*

*Trichomonas vaginalis* (figure 1-8) is usually identified based on vaginal, urethral or prostatic saline wet preparation examinations. Examine under low power with reduced illumination for actively motile organisms. Under high power, the undulating membrane may become visible as the organisms motility diminishes. Detection of the motile organism seems to lend itself to diagnosis, as stained smears tend to produce unreliable results.

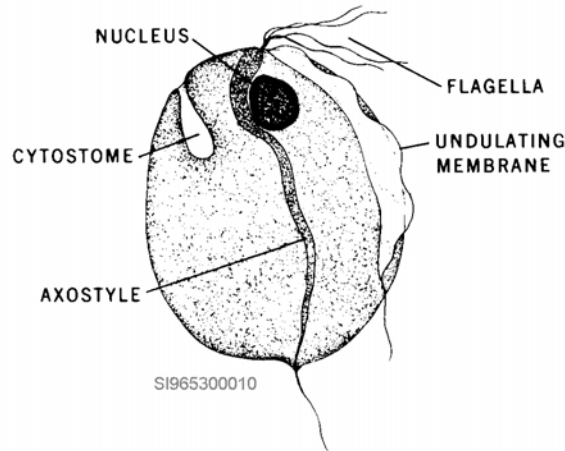


Figure 1-8. Drawing of *Trichomonas vaginalis*.

### *Aspirates*

Patients suffering from extraintestinal amebiasis can present a diagnostic challenge to the physician and laboratory. Stool samples will, in most instances, be negative for ameba, and serological tests have only limited usefulness in such cases since they remain positive for long periods after treatment of an earlier infection. In such cases, aspiration of abscesses is the best method for confirming a case of suspected extraintestinal amebiasis. Aspirates should be examined immediately. The basic procedures for examination are straightforward and uncomplicated. However, difficulties in detecting ameba aspirated from abscesses do arise. For example, liver and lung abscesses suspected of being caused by *E. histolytica* are sometimes drained, and the aspirate is examined for trophozoites. Organisms are located chiefly in the peripheral area of the abscess, and aspirates from the center portion are less likely to reveal trophozoites. Since trophozoites may be trapped in the thick pus, they may not exhibit typical motility. To overcome this difficulty, it may be helpful to treat the sample with a proteolytic enzyme, such as streptodornase, in order to free the organisms.

#### *Aspirates from abscesses*

The Amebiasis Research Unit, Durban, South Africa, recommends the following procedure be used in the collection of aspirates from abscesses. At least two portions of exudate should be removed and numbered. The first portion, usually yellowish-white, seldom contains amebae. However, later portions, reddish in color, are more likely to contain organisms, and the portion containing material from the near wall is most likely to be positive.

#### *Aspirates from cysts*

Beyond *E. histolytica* other organisms can be detected by aspiration, notably *E. granulosus*. The fluid from the aspirate is sent for laboratory examination to determine the presence of hydatid sand, which is collectively made up of degenerating scolices; scolices that bear hooklets and other distinctive forms. Some cysts are sterile, so this material may not be found in all aspirates even though disease exists. Aspirate from lymph nodes, spleen, liver, bone marrow, or spinal fluid may be examined for the presence of trypanosomes or leishmanial forms. Make a wet preparation to examine for motile

forms. Specimens from cutaneous lesions should be taken from deep under the ulcer for organism recovery since the surface may not contain the organism and may be contaminated with bacteria.

### ***Other body fluids and biopsy material***

Cerebrospinal fluid, bone marrow, blood, and biopsy material require immediate attention. It is imperative to process these samples immediately in order to prevent deterioration of any organism that is present. The following table is an overview of the infection site, specimen choices, collection methods, and the parasites commonly found at that site. Venipuncture, usually, refers to the immunological procedures that can be performed on serum. Diagnostic methods include direct wet mounts; Giemsa, Wright's, acid-fast, modified acid-fast, trichrome, modified trichrome, iron hematoxylin, and eosin stains; culture and tissue culture methods; electron microscopy; and immunospecific reagents and immunofluorescence staining (calcofluor). Refer to your department's operating instructions for the procedures used in your laboratory or for shipping instructions.

| <b>Infection Site</b>  | <b>Specimen Choices</b>                                                                                      | <b>Collection Methods</b>                                                                                | <b>Commonly Found Parasite</b>                                                                                                                                                        |
|------------------------|--------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Blood and Buffy coat   | Anticoagulated or whole blood                                                                                | Venipuncture or Capillary puncture                                                                       | <i>Plasmodium</i> , <i>Babesia</i> , <i>Trypanosoma</i> species, <i>Leishmania donovani</i> , <i>Toxoplasma gondii</i> , and microfilariae                                            |
| Bone marrow            | Bone marrow aspirate, serum                                                                                  | Sterile aspiration, venipuncture                                                                         | <i>Leishmania donovani</i>                                                                                                                                                            |
| Central nervous system | Spinal fluid, serum                                                                                          | Sterile CSF aspiration, venipuncture                                                                     | <i>Acanthamoeba/ Hartmannella</i> , <i>Naegleria</i> , <i>Echinococcus</i> , and <i>Trypanosoma</i> species, <i>Toxoplasma gondii</i> , <i>Taenia solium</i> , <i>Angiostrongylus</i> |
| Cutaneous ulcers       | Aspirate, biopsy, serum                                                                                      | Sterile aspiration/biopsy, pus smears, venipuncture                                                      | <i>Leishmania</i> species                                                                                                                                                             |
| Eye                    | Corneal scrapings                                                                                            | Sterile saline, air dried smear                                                                          | <i>Acanthamoeba</i> , and various microsporidia genera                                                                                                                                |
| Intestinal tract       | Fresh stool, preserved stool, sigmoidoscopy material duodenal contents, biopsy, serum, anal impression smear | Sterile container, Formalin, PVA, venipuncture, Entero-Test, aspirate, cellophane tape or pinworm paddle | Amoebas, microsporidia, pinworm, Helminths                                                                                                                                            |
| Liver and Spleen       | Aspirates, biopsy, serum                                                                                     | Sterile aspiration, venipuncture                                                                         | <i>Entamoeba histolytica</i> , <i>Leishmania</i> and <i>Echinococcus</i> species                                                                                                      |
| Lung                   | Sputum, lavage, transbronchial aspirate, brush biopsy, open lung biopsy                                      | Induced, no preservative, air dried smears, sterile aspiration                                           | <i>Pneumocystis carinii</i> , <i>Entamoeba histolytica</i> , <i>Paragonimus westermani</i> ,                                                                                          |
| Muscle                 | Biopsy, serum                                                                                                | Sterile procedure, venipuncture                                                                          | <i>Trichinella spiralis</i> , <i>Trypanosoma cruzi</i> , <i>Taenia solium</i> , <i>Onchocerca volvulus</i>                                                                            |
| Skin                   | Scrapings, biopsy, serum                                                                                     | Aseptic smear, sterile procedure, venipuncture                                                           | <i>Onchocerca volvulus</i> , <i>Leishmania</i> species, microfilariae                                                                                                                 |
| Urogenital system      | Vaginal and urethral discharge, prostatic secretions, urine, biopsy                                          | Saline swab, unpreserved urine specimen, sterile procedure                                               | <i>Trichomonas vaginalis</i> , <i>Schistosoma haematobium</i>                                                                                                                         |

**Accidental/incidental human parasites**

Often humans may serve as an accidental host to a parasite. Some examples will be explored here, and some may be similar in appearance to those that are human pathogens. You must be able to distinguish and differentiate between these accidental parasites and those that cause disease. Occasionally the parasitology department may be asked to examine specimens of non-human origin to rule in/out the source of an infection or to treat a military working dog as well as children in households with pets. Keep in mind that this is not an all-inclusive list, but some of the most commonly encountered.

***Dipylidium caninum***

*D. caninum* is the dog tapeworm that also sometimes afflicts felines. Unlike the eggs of other cestodes that are shed individually, *D. caninum* forms egg packets that may contain 5 – 30 eggs. The scolex of the tapeworm has a club shape with four suckers and six to seven circles of spines on the rostellum in lieu of hooks. The proglottids resemble pumpkin seeds. Both the characteristic egg packets and proglottids are diagnostic for this organism. Humans become accidentally infected through the ingestion of dog or cat fleas and when this occurs, replace the domesticated animals in the organism's life cycle. Most people will be asymptomatic due to a light worm burden. Those with heavier burdens can be treated with medications.

***Dirofilaria immitis***

This organism is the causative agent of dog heartworm (as well as other four legged mammals). The mosquito is the vector and humans are a dead end host because the organism cannot complete its life cycle in humans. The microfilaria of the organism will wander most often migrating to the lungs where it is trapped and becomes calcified forming what is called a coin lesion. To combat this, pet owners should treat their pets.

***Toxocara spp.***

*Toxocara* species (*Toxocara canis* and *T. cati*) usually affect children and is responsible for causing visceral larval migrans. The organism cannot complete its life cycle in humans and only the larval stage occurs. Children become infected from eating dirt or through poor handwashing hygiene. This allows for the ingestion of the eggs so it is often recommended that pets not be brought into the household until the child can read so as to avoid the crawling and toddler development stages where there is the most risk of infection. Dogs are diagnosed by finding the thick-shelled eggs that resemble a golf ball due to its pitted surface. Humans are diagnosed by clinical symptoms and identification of the larva from tissue samples. *Toxocara* can migrate to all areas of the body and proper identification is critical because of the detrimental effect the organism can have on the eye, resulting in blindness via ocular larval migrans in some cases when untreated.

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**Self-Test Questions**

After you complete these questions, you may check your answers at the end of the unit.

**601. Parasite classification and definitions**

1. Like the bacteria and fungi, all parasites are classified into what taxonomic designations?
2. What are the two subkingdoms of the parasites and what are their cellular characteristics?
3. Today, how are the parasites divided into their taxonomic designations?

4. What is an intermediate host?
5. What is a definitive host?
6. What is mutualism?
7. What parasites have a cyst and/or trophozoite stage in their life cycle?
8. What parasites have an egg and/or larva stage?

**602. Fecal specimen collection and preservation**

1. What are some substances that obscure or cause a decline in the number of protozoan organisms passed in a patient's feces?
2. How long after the use of barium will the detection of intestinal protozoa be impossible?
3. What impact may the contamination of feces with urine have on protozoan organisms that are present?
4. How many fecal specimens should be collected for a routine examination for parasites?
5. How many fecal specimens should be collected to diagnose a suspected case of amebiasis?
6. Within what time-frame should a series of three and six fecal specimens be collected?
7. How long after therapy should a specimen from a patient treated for helminths be examined?
8. How soon after collection should a liquid specimen for the detection of trophic amebas or flagellates be examined?

9. What are three accepted preservation methods?
10. What type of preservative is highly recommended for the preservation of protozoan cysts and trophozoites?
11. What is perhaps the greatest advantage of using PVA?
12. What preservative is recommended to prevent further development of helminth eggs?
13. What preservation solution uses sodium acetate to buffer formalin?
14. What are the four vials available in the collection kits?
15. What is the advantage of the collection kits?

**603. Other intestinal and nonintestinal specimens**

1. What is the most commonly used technique for the collection of *E. vermicularis* ova?
2. How many negative slides should be observed before the patient is considered free of *E. vermicularis* infection?
3. The collection of sigmoidoscopic material is usually associated with cases in which the patient is suspected of harboring what organism?
4. What is the objective of collecting sigmoidoscopic material?
5. What organisms are often found in duodenal drainage when stool specimens are negative?
6. Once obtained, how soon should duodenal contents be sent to the laboratory?



7. What is the easiest way of obtaining duodenal contents?
8. What type of motility is exhibited by *Giardia lamblia* trapped in mucus?
9. What phase of the life cycle of hookworms, *Ascaris lumbricoides*, and *Strongyloides stercoralis* accounts for their presence in sputum?
10. What are some of the other parasites recovered in sputum specimens?
11. What are some solutions that may be used to digest mucoid sputum specimens?
12. The presence of brownish flecks “iron filings” in sputum may be indicative of what organism?
13. How can *Entamoeba histolytica* be differentiated from *E. gingivalis* in sputum?
14. What are the two most common parasitic organisms found in urine and urogenital specimens?
15. How do you describe the optimal urine specimen for revealing eggs of *S. haematobium*?
16. How is *Trichomonas vaginalis* usually identified?
17. Why are serological tests for diagnosing extraintestinal amebiasis of limited usefulness?
18. What portion of an aspirated specimen for the diagnosis of extraintestinal amebiasis is most likely to be positive?
19. How do you describe the collection of a sample from a cutaneous ulcer for the recovery of leishmanial organisms?

20. What is the causative agent of dog heartworm?

## **1-2. Diagnostic Procedures**

The diagnosis of parasitic infections relies in large part on macroscopic and microscopic examination of feces, urine, blood, sputum, and biopsy material. Macroscopic examination can be used to determine the appropriate microscopic procedures to perform. The use of various microscopic examination methods can increase your chances of detecting and identifying a parasitic infection. In this section, it isn't possible to review more than a few of the commonly used laboratory procedures that can aid in the recovery and identification of parasitic forms in clinical specimens. For additional information on procedures, refer to the references listed in the bibliography.

### **604. Macroscopic examination of fecal specimens**

Two primary reasons exist for the visual examination of fecal specimens. First, it may reveal evidence of parasitic infection in the form of large diagnostic forms of parasites (adult worms, proglottids, or grossly abnormal appearance of the sample (blood, mucus, etc.). Second, it provides the opportunity to evaluate the consistency of the specimen and determines the diagnostic procedures most likely to yield positive results.

#### **Macroscopic evidence of infection**

Macroscopic evidence of infection is easy to determine and often is accomplished with the naked eye. For instance, adult roundworms or cestode proglottids may be found on the specimen surface. Sometimes these organisms will crawl under the specimen and will be found on the inside bottom of the container. This is especially true of *Ascaris* and the adult pinworm. Other roundworms may be seen in this manner but most often following medication.

You must always report the presence of blood in a stool specimen. When present, gross blood may present in forms, dark and fresh. Dark stools may be indicative of bleeding high in the gastrointestinal (GI) tract. Fresh blood (red) is usually caused by bleeding at a lower level in the GI tract. Any blood may be indicative of a parasitic or other pathologic process. Blood and/or mucus is seen in many intestinal parasitic infections and these areas should be carefully examined for amebic organisms. Fecal occult blood is not always indicative of a parasitic process and may be the symptom of other disease processes.

#### **Selection of procedures for fresh specimens**

The consistency of the sample is often a pivotal factor in determining what diagnostic procedures will be performed. We now consider two categories of samples:

1. Fresh.
2. Preserved.

Let's emphasize how the consistency of samples in either of these two categories can determine what diagnostic procedures will be performed. When fresh stool specimens are received in the laboratory, they should be examined macroscopically to determine their consistency. Four terms are generally sufficient to designate the consistency of the specimens:

1. Watery (W).
2. Loose (L).
3. Soft (S).
4. Formed (F).

Since fresh fluid specimens have the greater likelihood of containing protozoan trophozoites, they should be examined in the order listed. Figure 1-9 demonstrates the categories of consistency and the distribution of cysts and trophozoites. Distribution of eggs and larvae is less affected by the consistency of the feces.

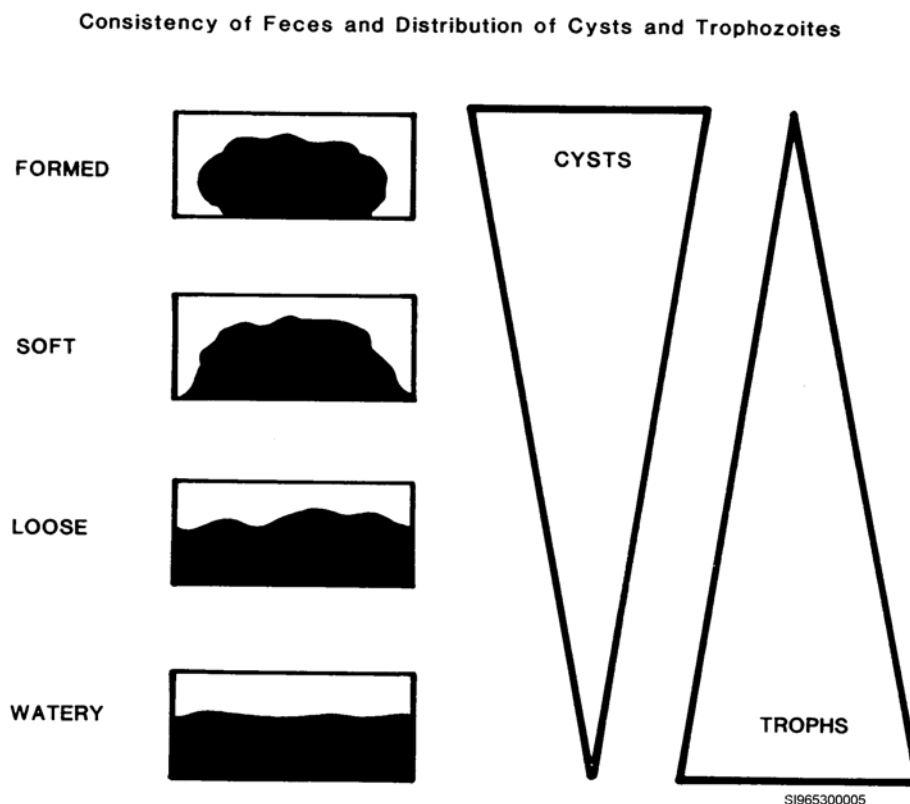


Figure 1-9. Consistency of feces and distribution of cysts and trophozoites.

### ***Watery or loose***

Specimens that are watery or loose are the most likely to contain protozoan trophozoites, which are readily detected by direct wet mounts and permanently stained smears due to their sheer numbers. Performing concentration techniques (except perhaps for simple centrifugation) is of little value since trophozoites are not demonstrated effectively by concentration techniques. Moreover, in this type of specimen, helminth eggs or larvae are usually present in sufficient numbers to be detected by the direct wet mount alone. However, if the direct wet mount and permanently stained smear fail to reveal an infection, it is usually advisable for you to proceed with a concentration technique.

### ***Soft***

For the examination of soft stools, wet mounts, concentrations, and permanent stains should be prepared since all forms of parasites may be present.

### ***Formed***

Following the macroscopic examination, formed stool specimens should be fully examined just as the softer counterparts for a variety of valid reasons. This includes a wet mount mount even though trophozoites are not typically observed in formed specimens. This ensures that some of the poorly concentrating parasites and parasite eggs such as those from *Hymenolepis nana*, *Giardia lamblia* do not concentrate well in the formalin-ether concentration procedure. Also, certain cestode eggs and the infertile eggs of *Ascaris lumbricoides* are not recommended in the zinc sulfate flotation method. The direct mount examination ensures that these diagnostic stages, if present, are observed.

### Selection of procedures for preserved specimens

Preservative solutions added to the specimen alter its original consistency and invalidate any grading system used by the laboratory. This problem is overcome if the laboratory uses a multi-vial system in which some of the original specimen is submitted without being altered in any way. This portion can then be used to grade consistency, and the appropriate procedures can be accomplished. If no such sample is available, the diluted sample is usually processed in the same manner as a loose or watery sample; that is, a direct wet mount is performed along with a permanently stained slide.

### The fecal occult blood test (Guaiac)

Fecal occult blood tests (FOBT) are often performed in the parasitology section of many laboratories. The FOBT is useful in the early detection of colorectal cancers. It is imperative that the laboratory provides the patient clear and unambiguous collection instructions to avoid repeated collections. To avoid erroneous results, the patient must follow specific collection procedures and many dietary restrictions during the course of specimen collection. The patient must especially avoid red meat because of the hemoglobin it contains as well as avoiding vegetables that contain peroxidase, which can falsely elevate the test results. Also, patients should discontinue medications that may interfere with the test if possible. Aspirin, which is used as a daily prophylaxis by many patients, is notorious for causing gastrointestinal bleeding, and it as well as other pain relievers (i.e.; NSAIDs) should be discontinued during the FOBT collection. Typically, specimen samples from three separate, but consecutive bowel movements are used. During specimen collection the patient must take care to avoid contamination of the stool with urine, water or soil. Women should not perform the collection either during or within three days of the completion of their menstrual period. The laboratory often provides a wide mouth container for collection of this specimen. Alternatively, the patient can line the toilet with plastic wrap held by the seat to collect the specimen. Normally individuals lose 2.0 to 2.5 mL of blood through the intestines daily. The FOBT detects occult blood losses greater than 5 mL per day. A small portion of stool is smeared on a FOBT card. The test employs the use of  $H_2O_2$ , which in the presence of peroxidase at the levels described above will yield a blue color indicating a positive result. Keep in mind that there are other methods available, but the card method described here is one of the most commonly used. Coming into more common use are flushable pads that conduct the test in the toilet and produce an observable color change if positive. Results are recorded on a card and mailed to the provider or laboratory. Hemorrhoids, anal fissures and other relatively benign conditions that cause digestive tract bleeding can lead to false positives.

| FECAL OCCULT BLOOD TEST DIETARY CONSIDERATIONS                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                   |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Patient Consumption Allowed                                                                                                                                                                                                                                             | Avoid Patient Consumption                                                                                                                                                                                                                                                         |
| Generous amounts of cooked and uncooked vegetables such as lettuce, corn, and spinach.<br>Moderate amounts of high fiber food such as bran cereal, peanuts, and popcorn.<br>Plenty of fruits such as apples, plums, and grapes.<br>Well cooked pork, poultry, and fish. | Rare and lightly cooked meats, particularly beef.<br>Cauliflower, horseradish, red radishes, turnips, broccoli, and cantaloupe.<br>Vitamin C in excess of 250 mg. per day.<br>Iron rich supplements.<br>Aspirin and other medications which may cause gastrointestinal irritation |
| <b>**REMEMBER:</b> Any and all changes to a medication/therapy regimen is/are directed by the patients attending health care provider, never by the laboratory.                                                                                                         |                                                                                                                                                                                                                                                                                   |

### 605. Microscopic examination of specimens

The following information explores the three most commonly performed procedures:

1. Direct wet mounts.
2. Direct wet mounts of concentrates.
3. Permanent stains.

It also includes the preparation and examination of blood smears for blood parasites.

### Preparation of direct wet mounts

Direct wet mounts are used to provide a quick diagnosis of heavily infected specimens, to check organism motility, and to diagnose organisms that might not be seen from concentration or permanent staining methods. Direct wet mounts should be prepared in both saline and iodine solutions. The direct wet mount procedure is reportedly of greatest value for observing motile protozoan trophozoites, a diagnostic stage that is unlikely to occur in formed specimens.

#### Saline

Emulsify a small portion of fecal material in a drop of physiologic saline on a microscope slide and overlaying the mixture with a cover slip to make the saline mount. Mounts should be just thick enough that newspaper print can be read through the slide. If the mounts are too thick, accurate observations cannot be made. If too thin, insufficient material may be available for examination. Saline mounts have the advantage of retaining the motility of trophozoites; however, definitive identification of either trophozoites or protozoan cysts in these preparations is difficult because the internal structures are often poorly visualized.

#### Iodine

Iodine is used to highlight the internal structures (nuclei and glycogen vacuoles) of any cysts that are present. One percent solutions of Dobell and O'Connor's, D'Antoni's, or Lugol's iodine should be used. Gram's iodine is *not* recommended for staining parasitic organisms. Iodine mounts alone, however, may not be satisfactory because the motility of trophozoites is often destroyed. Both types of mounts can be prepared and studied on a single microscope slide.

#### Procedure

Place a drop of physiological saline (0.85 percent NaCl) on one end of the slide and a drop of iodine (approximately 1 percent) on the other end as shown in figure 1-10.

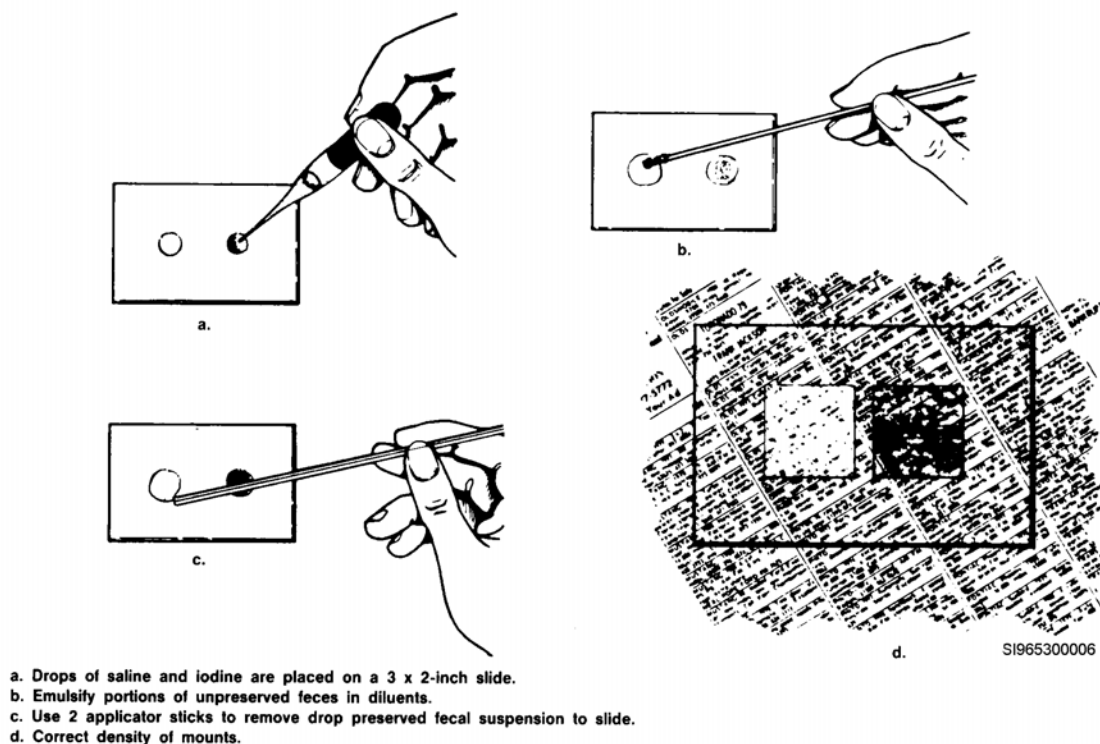


Figure 1-10. Preparing direct wet mounts of fecal material.

Using an applicator stick or toothpick, pick up a very small portion of feces and emulsify it in each solution. Keep the criteria for proper density in mind. Avoid large particles, if possible, since the coverslip will not lie evenly on the slide. Coverslip and examine the slide. If too little fluid has been used and the material does not fill up the coverslip area, additional saline or iodine may be run under the edge. The feces does not mix evenly with the diluent, of course, but the preparation can be examined more easily than if vacant spots were present. On the other hand, the coverslip should not float on the fecal suspension. In the latter situation, a piece of facial tissue or absorbent towel can be used to remove the excess fluid before sealing the preparation. Touch the tissue to the edges of the coverslip, but be careful not to remove too much fluid. Iodine preparations fade rather quickly if exposed to light; so, these mounts cannot be left indefinitely before being examined. If several preparations are made at one time, a slide folder can be used to hold them. Examine the entire smear under low power (100 X objective) and at least one-third of the slide under high dry power (400 X objective).

**NOTE:** The primary purpose of the direct wet mount is to see motility. Remember that once iodine is added to the preparation, the parasite will be killed and motility will be lost. Also, if the specimen is preserved, motility is lost. Therefore, many laboratories omit the direct wet mount, if only preserved specimens are received, and begin the stool examination with the concentration procedure. Results from direct wet mounts are preliminary and must be confirmed with concentration and permanent stain procedures.

### Microscopy

In parasitology, your microscope is your most important diagnostic tool. A good microscope is essential for examining parasitology specimens since the identification of organisms depends on the observation of morphologic details. The routine use of a *calibrated ocular micrometer* is also of great importance. Differentiating between the eggs of some helminths and the trophozoites of some protozoa may hinge on the use of a calibrated ocular micrometer. For example, the difference in size between the eggs of *H. nana* and *H. diminuta* is often used as the major criterion for differentiating the two species. Also, the pathogen *E. histolytica* is differentiated from the nonpathogenic protozoan *E. hartmanni* on the basis of size. Additionally, the use of a micrometer may help instill confidence in a technician who has limited parasitology experience by providing a concrete measurement on which to base his or her identification of an ova or trophozoite. Remember, some laboratory personnel, and physicians, too, may not be aware of the complexities diagnostic parasitology work entails. Yet no 2 by 2 inch color slide, line drawing, or verbal or written description can illustrate the tremendous morphologic variation seen in the diagnostic stages of the organisms, particularly the intestinal protozoa. There is just no substitute for actual “hands-on experience” at the bench.

**NOTE:** In diagnostic parasitology, “at the bench” often means at the microscope, and your proficiency is pivotal to the accurate diagnosis of parasitic infections.

### Concentration procedures

The basic purpose of all concentration procedures is to increase the chances of finding parasitic elements in fecal samples. Your twofold objective is to protect the microscopic morphology of the parasite, while decreasing the amount of fecal debris during the course of the concentration procedure. The two major concentration techniques are the following:

1. Flotation.
2. Sedimentation.

Both techniques have advantages and disadvantages in concentrating cyst, ova, and larvae of various parasites. To date, a practical means of concentrating protozoan trophozoites in fecal specimens has not been devised.

### ***Zinc sulfate flotation procedure***

The ability of certain helminth larvae and eggs, protozoan cysts, and coccidian oocysts of many parasites to float on the surface of a high specific gravity solution is at the heart of this procedure. These elements can be removed from the surface by using a Pasteur pipette or a wire loop. A zinc sulfate solution with a specific gravity of 1.18 is the most commonly used method. The primary advantage of flotation procedure is that it provides a very clean specimen for microscopic examination, that is, the amount of extraneous fecal debris remaining in the sample after processing is markedly decreased. However, this procedure is not recommended for samples containing large amounts of fatty materials or oils, since these materials will rise to the surface of the concentrate and make microscopic examination difficult. Also, this technique frequently fails to concentrate operculated ova and heavy eggs (for example, the unfertilized eggs of *Ascaris* species).

### ***Using a flotation procedure***

If you decide to use a flotation procedure as your primary or sole means of concentrating fecal samples, it is important that you keep the following points in mind.

1. The solution must be checked with a hydrometer.
2. Allow the centrifuge to stop without interference or vibration to prevent the organisms from being forced beneath the surface.
3. Ensure the detection of all organisms in the sample; carefully examine both the surface film and the sediment.
4. Limit the degree of distortion seen in any organisms that may be present, examine the film within 10 to 20 minutes after centrifugation.
5. If the specimen has been preserved with formalin, the specific gravity of the zinc sulfate used must be increased to 1.20.
6. Do not use the loop as a dipper by going below the surface of the centrifuged sample.
7. Procedural directions must be followed exactly to produce reliable results.

### ***Quality control***

Quality control for this procedure includes checking the specific gravity of the solution and purchasing formalin-fixed stools containing known parasites. Perform concentrations on quality control samples at least four times yearly. Refer to your laboratories' operating instruction for the exact procedure and quality control to be performed. Participating in survey programs can enhance the quality control of the laboratory data you produce. These survey programs offer you the chance to upgrade your proficiency by analyzing samples with known values. Your results are checked and compared with results from other laboratories, offering you the opportunity to gauge your own proficiency and the effectiveness of your procedures. As we noted earlier, proficiency in parasitology is growing in importance in the light of the increased mobility of our society.

### ***Formalin-ethyl acetate sedimentation procedure***

As the name implies, parasite concentration is achieved by allowing either gravity or centrifugation to separate the sample into layers, with most parasites being found in the bottom layer or sediment. A variety of detergents, organic solvents, and other reagents have been used to enhance the effectiveness of sedimentation procedures. A number of such organic solvents as ether, xylene, and ethyl acetate have been added to reduce the amounts of fats and oils in the sediment. Ethyl acetate has replaced ether and xylene for safety purposes. Detergents have been used to reduce the solution's surface tension and thus enhance parasite sedimentation.

### ***Advantage***

The primary advantage of the sedimentation technique is the ability to concentrate a larger variety of parasites than the flotation technique. For the most part, this is due to the fact that operculated and heavier ova are more readily recovered with sedimentation procedures. Also, because high specific

gravity solutions are not used, there is less chance that the parasites will be distorted. The two primary disadvantages are that protozoan cysts are not concentrated as effectively as with flotation procedures and fecal debris is present in larger amounts and may complicate interpretation. Also, *Hymenolepis nana* eggs may be missed, and concentrations of *Giardia lamblia* and *Iodamoeba bütschlii* cysts may be low.

#### *Most popular*

The most popular concentration technique of all is the formalin-ether concentration procedure of Ritchie. This procedure has been modified by substituting ethyl acetate for ether, thereby eliminating the handling of a potentially hazardous reagent without decreasing the effectiveness of the procedure. This procedure can be used on both fresh and preserved specimens. For example, this method, or slight modifications of it, may be used for processing specimens preserved in formalin (5 or 10 percent and buffered or non-buffered), SAF, or PVA. If a single technique is to be selected for routine use in your laboratory, this procedure is recommended. It is one of the easiest to perform and least subject to technical errors.

#### *Quality control*

Recommended quality control includes testing prepared known samples as mentioned for the zinc sulfate flotation procedure and monitoring proper centrifuge time and speed.

#### *Safety*

As always, the preeminent concern in the laboratory is safety. Concentration techniques that require the centrifugation of highly flammable reagents are a cause for concern. The primary health hazard of centrifuge accidents is their potential for creating large volumes of fine aerosol, which pose a definite threat to you and your coworkers; so always follow the manufacturers' operating instructions.

#### *Commercial systems*

There are several commercial systems available for concentration. These products are manufactured to work with the multi-vial collection kits.

### **Preparation of permanent stained smears**

Permanent stained smears provide contrasting colors for both the background debris and parasites present, recognition of detailed organism morphology under oil immersion (100 X objective), and enhanced recovery and identification of the intestinal protozoa. Convincing evidence of how permanent stains aid dramatically in confirming the presence of organisms is presented in this area. This is especially true of intestinal protozoa infections in which the main diagnostic form present, in the specimen, is the trophozoite. Also, information is included on what stain is best for a given specimen and its advantages and disadvantages.

### **Fecal smears**

The recovery and identification of intestinal protozoa often depends on the examination of a permanently stained smear. The definition and clarity of morphological details necessary for organism identification are frequently visible only in this type of preparation. An experienced microscopist can sometimes identify organisms on wet preparations, such as the direct smear or the concentration sediment. However, many of these identifications should be considered tentative until confirmed by the permanently stained smear. Many of the smaller organisms may be missed completely on examination of wet preparations, but they will be seen and identified on the permanently stained smear. For these reasons, the permanently stained smear is recommended for every stool specimen submitted for routine ova and parasite examination. Permanent stains can be done on fresh stool specimens and stool specimens preserved with Schaudinn's, PVA, SAF, or MIF, but *not* formalin.



***Most commonly used***

A number of staining methods are available; however, the two most commonly used are the following:

1. Iron hematoxylin.
2. Trichrome stains.

Both methods are excellent, and the choice can be based on the laboratory preference and past experience of those performing the test. The definitive morphology necessary for protozoan identification depends on adequate fixation within set timeframes after specimen passage. If these criteria are not met, the results will be less than adequate, regardless of the stain used.

***Iron hematoxylin***

Although there are many modifications of the longer Heidenhain iron hematoxylin method, a number of shorter methods can be used. Two of these are the Spencer-Monroe and Tompkins-Miller methods. These methods are somewhat more complicated than the trichrome method, and attention to protocol detail is necessary, particularly to the destaining step. A newer method adds a carbol fuchsin step in the procedure for the acid-fast organisms (for example *Cryptosporidium parvum*, *Cyclospora cayetanensis*, and *Isospora belli*). The modified procedure stains the background various shades of gray-blue, and the protozoa should be easily seen with medium-blue cytoplasm and dark blue-black nuclei.

***Trichrome***

The Wheatley modification of Gomori's original tissue trichrome method is recommended for general use. This method is highly reproducible and easy to perform. Consistently, good results can be obtained with both fresh and preserved specimens from PVA. This stain is generally not as good with SAF or MIF preserved specimens. Good reagents are available commercially, and they can be reused for a number of slides. The stain itself is very stable and can be used repeatedly. The background debris is green and the protozoa show a blue-green to purple cytoplasm. The nuclei and inclusions are red or purple-red and sharply outlined.

***Parameters that influence final results***

For laboratories that are not accustomed to doing permanent stains, it is important to realize many parameters influence the results. One such parameter is specimen age. Why? Because it is important to examine fresh specimens and place them in a preservative if they cannot be processed within an established time period. Another parameter is specimen consistency. This will influence the final stain color. Specimen composition is another parameter that will influence the result. But, most likely, the most important parameter is the lag time between passage of the specimen and fixation. The longer the specimen sits without preservative, the less chance that adequate organism morphology will be seen. Another important factor is smear preparation. As stated earlier, normally, the smear should be prepared so that newsprint can be read through it prior to staining. Staining reagents are also important and should be considered. Most of the reagents in the trichrome stain can be held in screwcap Coplin jars for some time. Those solutions that tend to require changing more frequently are the dehydration reagents. These include the 100 percent ethanol, xylene, and toluene.

***Guidelines***

As a reminder, many of the professional societies and published guidelines recommend that the permanent, stained slide should be a mandatory part of every ova and parasite examination performed on every specimen submitted to the laboratory. In the past, a misconception existed that the permanently stained smears do not need to be prepared on formed stool specimens. This is not correct; it is recommended that the permanent stain be performed on every stool specimen submitted for an ova and parasite examination.

***Quality control***

Slides with known parasites are commercially available and should be run along with patient samples using the same staining reagents and timing.

**Blood smears**

Because certain parasites can be found in whole blood and identification usually requires a permanent stain, blood films are required. The two types of smears are the thick and thin blood films. The films can be made from whole blood without anticoagulants, anticoagulated blood, or sediment from various concentration procedures.

**NOTE:** Pre-cleaned slides may not be very clean. An oily residue can persist on these slides, and it is advisable to rinse them in alcohol before using them for thick and thin blood films.

***Thick blood film***

To prepare a thick blood film, place two to three drops of fresh blood on an alcohol-cleaned glass slide. If possible, collect the blood from the fingertip without anticoagulant and spread it over the slide so that you have a dime-size spot that you can barely read newsprint through. Always request an ethylenediaminetetraacetic acid (EDTA) tube of blood to be used if the “fingertip” slides are of poor quality. The collection time should be annotated on the slides and/or tube of blood and on the final report for correlation of results with any fever pattern or other symptoms. Allow the slide to air-dry in a horizontal position for 12 to 24 hours. Never attempt to speed dry by applying heat to the smear because this will fix the RBCs and they will remain intact during staining. Hence, the result is stain retention and inability to identify the parasites.

***Advantages***

The major advantage of the thick film is that it allows a larger amount of blood to be examined, and thus hastens the screening of patient-stained samples. Thick films also increase the chances of detecting a light infection or one in its early stages. These two advantages are possible since a larger volume of blood is used to prepare the slide. One microscopic field of a thick film contains 15 to 25 times the amount of blood than that of a thin film.

***Disadvantages***

Because the blood is so thick, the microscopic morphology of the parasites may be distorted by this procedure. This makes thick films more difficult to read. They must be interpreted carefully due to the possible presence of debris or cellular components. Thus, this is a major disadvantage. The time required for air-drying is also a disadvantage.

***Thin blood film***

The thin blood film is used to reveal the morphological detail of the blood parasites. The thin film is prepared in the same manner as a smear prepared for the white blood cell (WBC) differential. The same criterion as to what constitutes a good smear for the WBC differential should also be applied to the thin film. The smear should provide an adequate area for examination where the cells are one layer thick, lying side by side but not overlapping, and evenly distributed. After the film air-dries, it is fixed (depending on staining procedure), and then stained.

**NOTE:** Automatic differential counters cannot identify parasites.

***Advantages***

The ability to reveal microscopic morphology is, of course, an advantage. Another advantage is the speed of processing because of its short drying time, which amounts to a matter of minutes as opposed to 12 to 24 hours for a thick film.

*Disadvantage*

The only real disadvantage is that the number of organisms per field is reduced compared to the thick film.

*Staining blood films*

Both thick and thin films can be stained with Giemsa or Wright's stain. Giemsa is preferred primarily because its color differentiation of parasitic components is better than that of Wright's stain. Giemsa stain also offers excellent resistance to fading and is readily available, in stock form, from a number of commercial sources. The major disadvantage of Giemsa staining is that it usually requires 20 to 50 minutes for staining versus 10 minutes for Wright's stain. In any event, smears should be stained as soon as possible after preparation (preferably within 30 minutes for thin smears).

**NOTE:** Giemsa stain is water-based; whereas, Wright stain is alcohol-based. This difference does effect the preliminary processing of smears. When working with thin blood films (to be stained by Giemsa) they must be fixed in absolute methanol before staining; whereas thick smears do not require this pretreatment because the cells will be lysed during staining. Also, when an alcohol-based stain is used, thin smears require no pretreatment, but thick smears must be lysed in either buffered water or a saponin reagent. When slides are removed from staining reagents, allow to air dry vertically.

*Giemsa staining results*

Giemsa stain colors the blood components as follows:

- RBCs are pale red.
- WBC nuclei are purple with a pale purple cytoplasm.
- Eosinophilic granules are bright purple-red.
- Neutrophilic granules are deep pink-purple.

If malaria parasites are present, the cytoplasm stains blue and the nuclear material stains red to purple-red. Schüffner's dots and the RBC inclusions will stain red. Nuclear and cytoplasmic staining characteristics of the other blood parasites such as *Babesia* species, trypanosomes, and leishmaniae will stain like those seen in the malaria parasites. While the sheath of microfilariae may not always stain with Giemsa, the nuclei within the microfilaria itself will stain blue to purple.

*Wright's staining results*

Wright's stain colors the blood components as follows: RBCs are buff, light tan, or reddish; WBC nuclei bright blue with a contrasting light cytoplasm; eosinophilic granules are bright red; neutrophilic granules are pink to a light purple. If malaria parasites are present the cytoplasm stains a pale blue and the nuclear material stains red. Schüffner's dots and other RBC inclusions do not stain or stain very pale. Nuclear and cytoplasmic staining characteristics of the other blood parasites such as *Babesia* species, trypanosomes, and leishmaniae will stain like those seen in the malaria parasites. While the sheath of microfilariae may not always stain with Wright's stain, the nuclei within the microfilaria itself will stain pale to dark blue.

*Examining blood films*

Examine approximately 100 fields under oil immersion (100 X objective) for thick blood films. Initial scanning of thin smears under low power is suggested for the detection of larger parasites, such as microfilariae and trypanosomes. The parasites that are smaller and more difficult to detect and identify, such as malaria, demand the use of the 100 X oil immersion objective (1,000 times total magnification). At least 200 fields are examined for thin blood films.

**Buffy coat films**

Prepare buffy coat films by first centrifuging whole citrated or oxalated blood and then removing the buffy coat with a Pasteur or capillary pipette. The buffy coat can be examined as a direct wet mount

by mixing 0.5 drop of saline with 1 drop of buffy coat sediment on a slide and coverslipping. Examine under low power (10 X objective) for motile trypomastigotes and microfilariae. Prepare a thin film with the buffy coat (same as blood), allow to air dry, and proceed with either the Giemsa or Wright's staining procedure.

### **Artifacts and/or pseudoparasites**

Any items can resemble parasites in both fecal and blood film exams. Some of these include air bubbles, crystals, mites, plant hair and fibers, pollen, yeast, mucus casts, free-living nematodes, PMNs and other cells, synthetic fibers, fungal elements among others. You must be able to distinguish parasites from artifacts with certainty. There are numerous parasite textbooks and websites with more information. Of special note are Charcot-Leyden crystals which are formed from the breakdown of eosinophils and can be indicative of parasitic infection and may be seen in the stool.

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## **Self-Test Questions**

**After you complete these questions, you may check your answers at the end of the unit.**

### **604. Macroscopic examination of fecal specimens**

1. What are some examples of macroscopic evidence of a parasitic infection?
2. What may a dark stool sample indicate?
3. What type of stool sample may be highly suggestive of an amebic infection?
4. Why is it important to note the consistency of a fecal sample?
5. What four terms are used to describe fecal consistency?
6. Referring back to figure 1-9, what type of specimen is most likely to contain trophozoites?
7. What eggs are not effectively concentrated by the zinc sulfate flotation method?
8. How may the problem, of a specimen's consistency being altered by the addition of preservative solutions, be overcome?
9. How much blood loss is detectable by FOBT?

10. How many specimens are collected to detect fecal occult blood?

**605. Microscopic examination of specimens**

1. What are the purposes of the direct wet mount?
2. What two solutions are commonly used to prepare direct wet mounts?
3. How thick should wet mounts be prepared if accurate microscopic observations are to be made?
4. What advantages and disadvantages do saline mounts have over iodine mounts?
5. What type and concentration of iodine is used in wet mount preparations?
6. What two objectives should you try to achieve during the course of a fecal concentration procedure?
7. What are the two major concentration techniques?
8. What is the primary advantage of flotation procedures?
9. What are some disadvantages of flotation procedures?
10. What type of substances have been used to reduce the amount of fats and oils in sedimentation procedures?
11. What is the primary advantage of sedimentation procedures?
12. What are the two primary disadvantages of sedimentation procedures?

13. What is the recommended quality control for both procedures?
14. The identification of what type of organism is often dependent on a permanently stained smear?
15. What are the two most commonly used stains for the preparation of permanently stained fecal smears?
16. What methods for permanently stains of fecal material are suggested for general use?
17. What are some parameters that influence the results of a permanent stain?
18. On what type of stool specimen is it recommended that you perform permanent stains?
19. What are the two types of blood films used to identify blood parasites?
20. What type of blood specimens can be used for making these blood films?
21. What are the advantages of the thick film?
22. What is a disadvantage of the thick film?
23. What are advantages of the thin film?
24. What are the two primary stains used for thick and thin films?
25. What organisms can be seen in a direct wet mount of a buffy coat?

## Answers to Self-Test Questions

### 601

1. Kingdom, phylum, class, order, family, genus, and species.
2. Subkingdom *Protozoa*-unicellular organisms and the subkingdom *Metazoa*-multicellular organisms.
3. The early systematists divided organisms based on their macroscopic or microscopic descriptions. Today, they rely on these published species descriptions, as well as on studies of DNA, proteins, ecological function, and geographical distribution to develop phylogenies of parasites.
4. The *intermediate host* is required in the life cycle of the parasite for larval development. This development must occur before the parasite is infectious to the definitive host or the secondary intermediate host.
5. The *definitive host* is where the sexual reproduction of a parasite takes place.
6. *Mutualism* is an association in which both organisms benefit and cannot survive without each other.
7. Amebae, flagellates, or ciliates.
8. Helminths or worms.

### 602

1. Radiologic reagents, mineral oil, bismuth, nonabsorbable antidiarrheal preparations, antimalarials, and some antibiotics.
2. At least 1 week.
3. They may lose their motility or undergo lyses.
4. Three.
5. Six.
6. 10 days; 14 days.
7. 5 to 6 weeks.
8. Within 30 minutes after passage.
9. (1) Holding the specimen at room temperature if it can be examined within 2 or 3 hours.  
(2) Holding the specimen at refrigerator temperature for a maximum of 2 to 3 days.  
(3) Placing the specimen in one or more of various preservative solutions.
10. PVA.
11. A permanent, stained slide can be prepared from PVA-preserved material.
12. 5 to 10% or hot 5% formalin (60°C).
13. SAF.
14. Plain clean vial, Zn-PVA, 10% buffered neutral formalin, and Cary Blair transport media.
15. The collection kits reduces the lag time between when the specimen is passed and when it is fixed.

### 603

1. Cellophane tape or pinworm paddle method.
2. Four to six.
3. *Entamoeba histolytica* or other amebae.
4. To obtain material from the mucosal surface of the large intestine.
5. *Strongyloides stercoralis* and *Giardia lamblia*.
6. Within 1 hour.
7. Entero-Test or string test.
8. Rapid fluttering of the flagella.
9. Their migration through the lungs.
10. The trophozoites of *Entamoeba histolytica*, *Entamoeba gingivalis*, and *Trichomonas tenax*; oocysts of *Cryptosporidium parvum*; and *Pneumocystis carinii* (now classified as a fungi); can also be seen in sputum specimens. On rare occasions, the immature protoscolices or hooklets of *Echinococcus granulosus*.
11. Undiluted bleach or 3% sodium hydroxide.

12. *Paragonimus westermani*.
13. *E. gingivalis* will contain ingested polymorphonuclear neutrophils, but *E. histolytica* will not.
14. *Trichomonas vaginalis* and *Schistosoma hematobium*.
15. Last portion of a midday specimen collected after 10 to 15 minutes of strenuous exercise.
16. The identification of *Trichomonas vaginalis* is usually based on the examination of wet preparations of vaginal and urethral discharges, and prostatic secretions. These specimens are diluted with a drop of saline and examined under low power, with reduced illumination, for the presence of actively motile organisms; urine sediment can be examined in the same way.
17. They remain positive for long periods after treatment.
18. The portion containing material from the near wall of the abscess.
19. Samples aspirated from below the ulcer bed.
20. *Dirofilaria immitis*.

**604**

1. Adult worms, proglottids, and gross blood or mucus.
2. Bleeding high in the intestinal tract.
3. A soft or liquid stool.
4. It is often a pivotal factor in determining what diagnostic procedures will be performed on the specimen.
5. Watery, loose, soft, and formed.
6. Watery.
7. Certain trematode eggs and infertile *A. lumbricoides* eggs.
8. By using a multi-vial system in which some of the original specimen is submitted without being altered in any way.
9. Greater than 5mL/day.
10. Three distinct and separate specimens.

**605**

1. Direct wet mounts are used to provide a quick diagnosis of heavily infected specimens, to check organism motility, and to diagnose organisms that might not be seen from concentration or permanent stain methods.
2. Physiologic saline and iodine.
3. Just thick enough so that newsprint can be read through the slide.
4. Trophozoites retain their motility; the internal structures of cysts and trophozoites are difficult to identify in saline wet mounts.
5. One percent solutions of Dobell and O'Connor's, D'Antoni's, or Lugol's iodine.
6. Protect the microscopic morphology of the organisms you are attempting to recover, while decreasing the amount of fecal debris present in the sample.
7. Flotation and sedimentation.
8. They provide you with a very clean specimen to examine microscopically.
9. Not recommended for samples containing large amounts of fatty materials operculated or heavy eggs.
10. Organic solvents, detergents, and other reagents.
11. Its ability to concentrate a larger variety of parasites than the flotation technique.
12. Protozoan cyst are not concentrated as effectively as with flotation procedures, and fecal debris are present in larger amounts.
13. Recommended quality control includes testing prepared known samples.
14. Intestinal protozoa.
15. Iron hematoxylin and trichrome.
16. The Wheatley modification of the Gomori's original tissue trichrome method.
17. Specimen age, preservation, consistency, composition, smear preparation, and reagent quality.
18. It is recommended that permanent stains should be performed on all specimens.



19. Thick and thin blood films.
20. The films can be made from whole blood without anticoagulants, anticoagulated blood, or sediment from various concentration procedures.
21. The major advantage of the thick film is that it allows a larger amount of blood to be examined, and thus hastens the screening of patient-stained samples. Thick films also increase the chances of detecting a light infection or one in its early stages.
22. Because the blood is so thick, the microscopic morphology of the parasites may be distorted by this procedure.
23. The ability to reveal microscopic morphology is, of course, an advantage. Another advantage is the speed of processing because of its short drying time, which amounts to a matter of minutes as opposed to 12 to 24 hours for a thick film.
24. Giemsa and Wright's stain.
25. Motile trypomastigotes and microfilariae.

**Do the unit review exercises before going to the next unit.**

## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter. When you have completed all unit review exercises, transfer your answers to ECI (AFIADL) Form 34, Field Scoring Answer Sheet.

**Do not return your answer sheet to AFIADL.**

1. (601) The active, motile, feeding stage of a protozoa is the
  - a. cyst.
  - b. larva.
  - c. cercariae.
  - d. trophozoite.
2. (602) How long after treatment for a *Taenia* infection is a patient's specimen examined to determine the effectiveness of the treatment?
  - a. 3 days.
  - b. 1 to 2 weeks.
  - c. 3 to 4 weeks.
  - d. 5 to 6 weeks.
3. (602) The preservative solution that can **only** be used for concentration procedures and wet mounts but **not** for permanently stained smears is
  - a. formalin.
  - b. polyvinyl alcohol.
  - c. merthiolate-iodine-formalin.
  - d. sodium acetate-acetic acid-formalin.
4. (603) The presence of *hydatid sand* in a sputum specimen is indicative of
  - a. *Paragonimus westermani*.
  - b. *Echinococcus granulosus*.
  - c. *Strongyloides stercoralis*.
  - d. *Entamoeba histolytica*.
5. (603) In a watery or loose specimen causative organisms are usually present in numbers that allow detection by
  - a. Methiolate-iodine formalin (MIF).
  - b. Polyvinyl alcohol (PVA).
  - c. zinc sulfate floatation.
  - d. wet mount.
6. (603) The portion of a specimen aspirated from a liver or lung abscess and *most* likely to be positive is the
  - a. center portion.
  - b. thickest, yellowish-white portion.
  - c. portion aspirated from the near wall of the abscess.
  - d. portion aspirated from around the exterior of the abscess.
7. (604) Zinc sulfate flotation is **not** recommended for concentrating heavy eggs such as the
  - a. fertile eggs of *Ascaris* and certain cestode eggs.
  - b. fertile eggs of *Ascaris* and certain nematode eggs.
  - c. infertile eggs of *Ascaris* and certain nematode eggs.
  - d. infertile eggs of *Ascaris* and certain trematode eggs.

8. (604) Which of the following is **not** a source of error in the fecal occult blood test?
- a. Poultry and vitamin E consumption.
  - b. Beef and Vitamin C consumption.
  - c. Aspirin ingestion.
  - d. Hemorrhoids.
9. (605) The stool specimen that is **least** likely to contain protozoan trophozoites is
- a. soft.
  - b. loose.
  - c. watery.
  - d. formed.
10. (605) The solutions used to prepare direct wet mounts are
- a. saline and iodine.
  - b. formalin and ethyl acetate.
  - c. three percent NaOH and PVA.
  - d. undiluted bleach and 3 percent N-acetyl cystine.
11. (605) What iodine solution is **not** recommended for staining parasitic organisms in a direct wet mount?
- a. Gram.
  - b. Lugol.
  - c. Dobell.
  - d. D'Antoni.
12. (605) The two most commonly used stains for preparing permanent stains of fecal smears are
- a. Spencer and Abbott.
  - b. De Tomasi and Coleman.
  - c. Dobell's and D'Antoni's.
  - d. iron hematoxylin and trichrome.
13. (605) What staining technique is generally used in staining fecal smears?
- a. Wheatley's modification of the Gomori trichrome method.
  - b. Tompkins-Miller's iron hematoxylin.
  - c. Heidenhain's iron hematoxylin.
  - d. Spencer's iron hematoxylin.

## **Student Notes**

## Unit 2. Lumen-Dwelling Helminths

|                                                                                                    |             |
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**I**N this unit you will study lumen-dwelling helminths; that is, helminths (roundworms, tapeworms, and flukes) that live in the canal, duct, or cavity of a tubular organ. These organisms vary in size from millimeters to 10 meters in length. In order to study these various parasites in an organized manner, you'll see a common format. This format consists of the four headings of *life cycle*, *epidemiology*, *pathology and clinical manifestations*, and *laboratory diagnosis*.

Helminth *life cycles* can be direct or indirect. Direct development requires only one host that harbors the adult worms, eggs, or larvae that are passed in the stool. In some instances, the eggs are infective when passed, while in others they require a soil maturation period to reach the infective stage. Indirect cycles are more complex and include intermediate hosts (where the larval stages develop) and definitive hosts (which harbors the adults). Adult and larval stages occur in intestinal lumen or tissues of the host. Most living organisms, including humans, serve as definitive and/or intermediate hosts for many parasitic helminths.

*Epidemiology*, the study of the frequency and distribution of disease, can contribute significantly to your success in parasitology. Many comprehensive textbooks of parasitology, however, present animal parasites as if the diseases they cause occur with equal frequency. As a parasitology student, you must have a broad knowledge of parasitology, but you should place increased emphasis on those organisms most likely to be encountered at your current duty station. Knowing what organisms are most prevalent in your geographic area can narrow your search and speed the laboratory diagnosis of a parasitic infection.

The *pathology and clinical manifestations* associated with various parasitic infections are helpful to you as further aids in the diagnosis of parasitic disease. Signs and symptoms of helminth infections are caused by adult worms, larvae, and/or eggs, and, possibly, the hosts reaction to the parasites. Eosinophilia is common, especially in early stages of infection in which parasites are in tissue.

If a parasitic infection is to be *diagnosed*, a clear understanding of the life cycle, epidemiology, and pathology of the organism is essential. Final *diagnosis* usually depends on the morphologic identification of a parasitic stage (egg, larva, or adult) in feces, blood, sputum, urine, or tissue. However, in some infections, only a clinical diagnosis is possible; or diagnosis is established indirectly by immunological methods. These methods include indirect hemagglutination (IHA),

enzyme immunoassays (EIA), indirect immunofluorescence test (IFA), complement fixation (CF), immunoblot (IB) assays, bentonite flocculation, and DNA-based molecular methodologies. Of course, these techniques are not used in the identification of all parasites and are in different stages of development for practical use. The subsequent lessons will focus on macroscopic and microscopic morphology identification.

## 2–1. Nematodes (Roundworms)

Intestinal nematodes *typically* do not have an intermediate host in their life cycle. All nematodes, except *Strongyloides* have two sexes and characteristically have four larval stages and an adult stage. The eggs or larvae are passed in feces and require a period of development outside the host to reach the infective stage. The life cycles of the intestinal nematodes vary in complexity from the very simple pattern of *Enterobius* to the involved pattern of *Strongyloides*.

### 606. *Enterobius vermicularis* (pinworm)

It is thought that *Enterobius vermicularis* causes the most common human parasitic infection in the world. The disease caused by *E. vermicularis* is known as enterobiasis, pinworm infection, and oxyuriasis.

#### Life cycle

The pinworm benefits from a simple life cycle as illustrated in figure 2–1. Infection occurs when eggs containing third-stage larvae are ingested. After hatching in the upper small intestine, the larvae migrate to the ileum and cecum regions of the intestine where they reach maturity. The adult worms attach themselves to the mucosa, where they feed on bacteria and epithelial cells and become sexually mature in 15 to 43 days after the infection occurs. Shortly after fertilization, the gravid female migrates from the cecum to the perianal region, where she expels her eggs and *usually* dies. In females, she may migrate into the vagina, uterus, or fallopian tubes and die.

#### Causal Agent

The nematode (roundworm) *Enterobius vermicularis* (previously *Oxyuris vermicularis*) also called human pinworm (adult females: 8 to 13 mm, adult male: 2 to 5 mm.). Humans are considered to be the only hosts of *E. vermicularis*. A second species, *Enterobius gregorii*, has been described and reported from Europe, Africa, and Asia. For all practical purposes, the morphology, life cycle, clinical presentation, and treatment of *E. gregorii* is identical to *E. vermicularis*.

Refer to figure 2–1 for the following references. Eggs are deposited on perianal folds ❶. Self-infection occurs by transferring infective eggs to the mouth with hands that have scratched the perianal area ❷. Person-to-person transmission can also occur through handling of contaminated clothes or bed linens. Enterobiasis may also be acquired through surfaces in the environment that are contaminated with pinworm eggs (e.g., curtains, carpeting). Some small number of eggs may become airborne and inhaled. These would be swallowed and follow the same development as ingested eggs. Following ingestion of infective eggs, the larvae hatch in the small intestine ❸ and the adults establish themselves in the colon ❹. The time interval from ingestion of infective eggs to oviposition by the adult females is about one month. The life span of the adults is about two months. Gravid females migrate nocturnally outside the anus and oviposit while crawling on the skin of the perianal area ❺. The larvae contained inside the eggs develop (the eggs become infective) in 4 to 6 hours under optimal conditions ❶. Retroinfection, or the migration of newly hatched larvae from the anal skin back into the rectum, may occur but the frequency with which this happens is unknown.

#### Geographic Distribution

Worldwide, with infections more frequent in school or preschool children and in crowded conditions. Enterobiasis appears to be more common in temperate than tropical countries. The most common helminthic infection in the United States (an estimated 40 million persons infected).

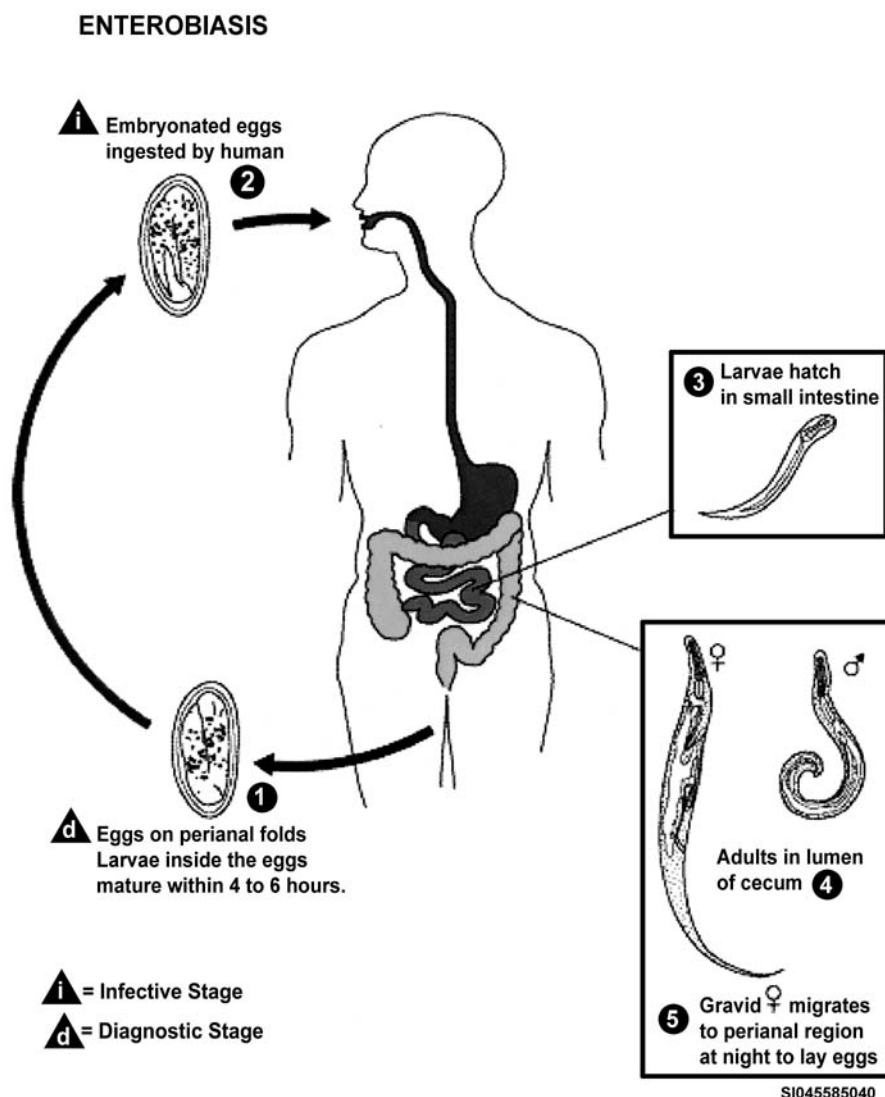


Fig. 2-1. Life cycle of *Enterobius vermicularis*.

The male dies soon after fertilization of the female and is passed, usually unnoticed, with the feces. The eggs *in utero* are not fully embryonated; however, once they are discharged from the female they rapidly mature to contain fully infective larvae within about 6 hours from the time they are deposited. Each female can deposit from 4,000 to 16,000 eggs that stick to the perinatal folds and may survive as long as 10 days in a humid environment. Under the right conditions, reinfection is possible. An unusual route of reinfection occurs when larvae hatch from the eggs deposited on the perianal folds, migrate into the anus, and mature into adult worms in the intestine.

### Epidemiology

*Enterobius vermicularis*, is worldwide in distribution, but is more common in cool or temperate regions than in strictly tropical areas. It is the most common nematode infecting humans in the United States. It is most frequently found in small children who live in crowded conditions. The most common route of infection occurs when infective eggs are ingested from contaminated fingers or fomites (for example, bed cloths, bed linen). Dust-borne infection can also occur when individuals inhale the lightweight eggs that become airborne. Once initiated, the chain of infection and reinfection can be extremely difficult to break. It is prudent to treat all members of a household, while encouraging them to disinfect their living quarters thoroughly. Personal hygiene, with emphasis on frequent hand washing, should also be stressed.

**Pathology and clinical manifestations**

Clinically, in approximately one-third of all pinworm infections, the patient is asymptomatic. In the majority of all cases, the symptoms are mild. When they do occur, the two symptoms are usually localized itching and behavioral changes. The localized symptoms are in the form of *pruritus ani* (intense chronic itching in the anal region). The behavioral changes include insomnia, irritability, nervousness, inattention, lack of cooperation, and poor appetite. Both the pruritus and the behavioral changes are probably due to absorption of metabolites secreted by the worm and subsequent development of hypersensitivity to these metabolites. Occasionally, females will migrate to unusual sites such as the uterus, fallopian tubes, or peritoneal cavity. When this occurs, a variety of manifestations, including vulvovaginitis, salpingitis, and pelvic granuloma, have been described.

**Laboratory diagnosis**

Finding characteristic eggs or an occasional adult female on cellulose tape preparations (discussed in unit 1) taken from the perianal skin provide diagnosis of the infection. The specimen should be collected in the early morning, before the patient showers or has a bowel movement. Four to six specimens, collected on successive mornings, should be taken before enterobiasis is ruled out.

**Eggs**

The eggs are elongated with a thick colorless shell, flattened on one side, range in size from 50 to 60  $\mu\text{m}$  long by 20 to 40  $\mu\text{m}$  wide, and contain infective larvae.

**Adult worms**

Adult pinworms are cylindrical in shape and whitish in color. As with most nematodes, the male is smaller than the female. The male is about 2 to 5 mm long, while the female is approximately 8 to 13 mm long and has a long, straight, sharply pointed posterior that gives this nematode its common name, pinworm. The posterior end of the male is sharply curved so that its body resembles an upside down question mark.

**607. *Trichuris trichiura* (whipworm)**

*Trichuris trichiura* is seen more frequently in older children because of the longer life span of the adult worms, which can live for 10 years or longer. The disease caused by *T. trichiura* is known as trichuriasis or whipworm infection.

**Life cycle**

The life cycle of *Trichuris trichiura* is similar to that of *E. vermicularis*; however, the eggs have to embryonate for 3 weeks after passage outside the body to be infective. The infection is acquired by ingestion of embryonated eggs that hatch and mature into worms in the cecum and less commonly in the walls of the large intestine, appendix, and lower part of the ileum. Eggs appear in the feces about 90 days after ingestion of the embryonated eggs.

**Epidemiology**

The whipworm has a worldwide distribution, but it is more common in temperate or tropical climates. Patients infected with *Trichuris* are frequently found to harbor coexisting infections with *Ascaris* and/or hookworm. The mode of transmission is indirect and not immediately transmissible from person-to-person.

**Pathology and Clinical Manifestation**

Light infections do not usually cause symptoms. The presence of moderate to large numbers of worms causes bloody diarrhea. Heavy infections result in malnutrition and dysentery with bloody mucoid stools. *T. trichiura* dysentery may have to be differentiated from that caused by *Entamoeba histolytica*. Rectal prolapse may occur in heavily infected children. In direct saline smears from cases involving moderate to heavy infection you will find eggs, many eosinophils, and Charcot-Leyden



crystals. The eosinophils and Charcot-Leyden crystals may even be present several weeks before the worms start to produce eggs.

### Laboratory diagnosis

Diagnosis is made by demonstration of eggs in feces or by sigmoidoscopic observation of worms attached to the wall of the lower colon in heavy infections.

### Eggs

Eggs of *Trichuris* passed in stool specimens are yellowish-brown, thick shelled, and barrel-shaped, with clear, mucoid polar plugs at each end. The eggs maintain their characteristic shape even on permanent, stained smears. The barrel-shaped egg, as shown in figure 2-2, laid in the one cell stage, is 50 to 55  $\mu\text{m}$  long 22 by to 24  $\mu\text{m}$  wide. Egg quantification is essential to relate the infection to clinical signs and symptoms, since light infections do not cause detectable disease and probably would not be treated. The laboratory report should include the terms; rare, few, moderate, or many for quantification. After mebendazole treatment, some patients may continue to pass *T. trichiura* eggs. In many instances, these eggs will be distorted and malformed.

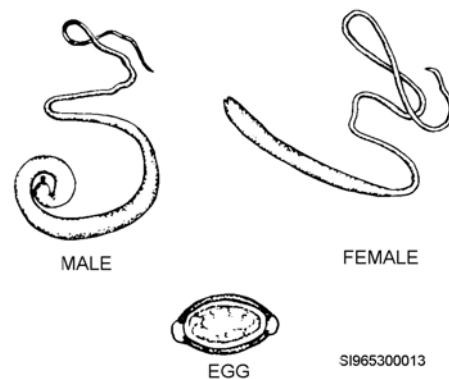


Fig. 2-2. *Trichuris trichiura* adult worms and egg.

### Adult worms

Adult worms may be encountered in stool specimens from heavily infected patients. The anterior two-thirds of the worm is slender and threadlike; whereas the posterior one-third is thick and fleshy. *In vivo*, the anterior of the worm is delicately but firmly threaded into the mucosal epithelium of the cecum. The male measures 30 to 45 mm in length. Its posterior end is heavyset and curled into a full circle. The female measures 35 to 50 mm in length. Its body is bluntly rounded at the posterior end. The female *T. trichiura* has only one ovary and produces relatively few eggs (3,000 to 6,000 per day).

### Causal Agent

The nematode (roundworm) *Trichuris trichiura*, also called the human whipworm.

Refer to fig. 2-3 for the following references. The unembryonated eggs are passed with the stool ❶. In the soil, the eggs develop into a 2-cell stage ❷, an advanced cleavage stage ❸, and then they embryonate ❹; eggs become infective in 15 to 30 days. After ingestion (soil-contaminated hands or food), the eggs hatch in the small intestine, and release larvae ❺ that mature and establish themselves as adults in the colon ❻. The adult worms (approximately 4 cm in length) live in the cecum and ascending colon. The adult worms are fixed in that location, with the anterior portions threaded into the mucosa. The females begin to oviposit 60 to 70 days after infection. Female worms in the cecum shed between 3,000 and 20,000 eggs per day. The life span of the adults is about 1 year.

## TRICHURIASIS

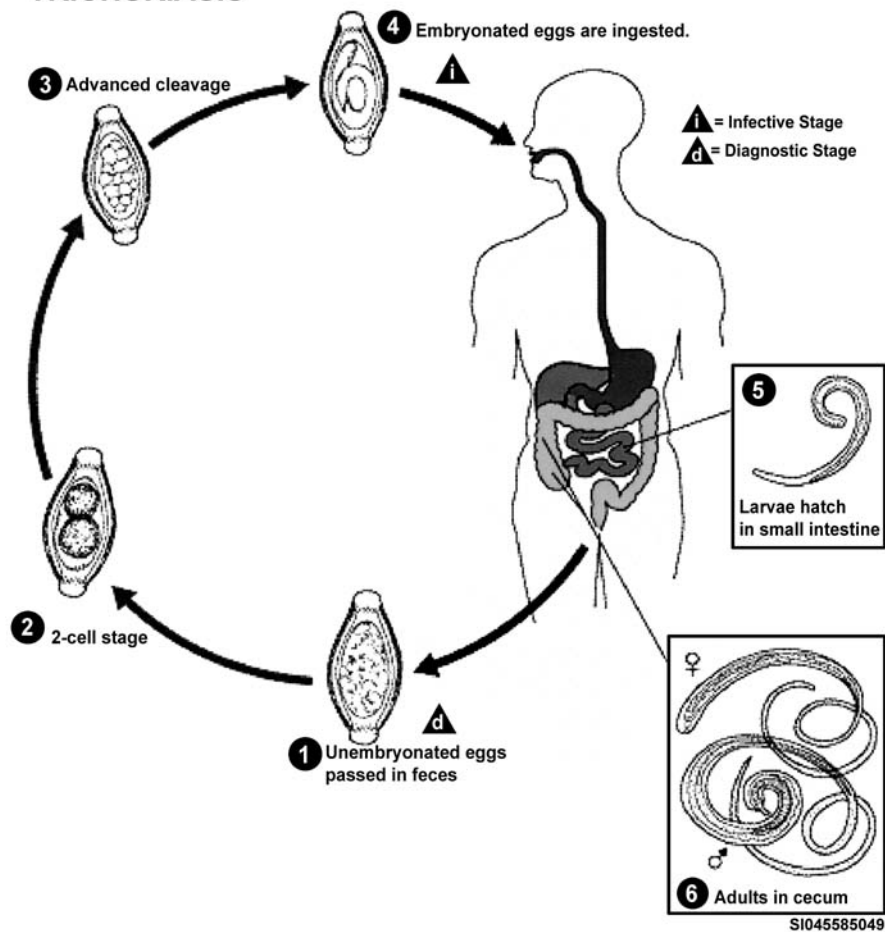


Fig. 2-3. *Trichuris trichiura* life cycle.

### Geographic Distribution

*Trichuris trichiura* is the third most common round worm found in humans. Worldwide, infections are more frequently found among children, in areas with tropical weather and poor sanitation practices. It is estimated that 800 million people are infected worldwide. Trichuriasis occurs in the southern United States.

### *Capillaria philippinensis* overview

*Capillaria philippinensis* was initially restricted to the Philippines and Thailand. It was first recognized in the mid-1960s as causing human infection known as intestinal capillariasis or hepatic capillariasis. Recently, cases of infection have been reported in Japan, Taiwan, Egypt, Iran, and Indonesia. The life cycle details are not clearly known, but human infection occurs from the ingestion of raw fish. The infective larvae are located in the mucosa of the fish intestine. The eggs require 10 to 14 days in the soil to embryonate and 3 weeks to develop into the infective form in fish. Symptoms include weight loss, muscle wasting, abdominal distention, and edema. Death can occur because patients develop pneumonia, heart failure, hypokalemia, or cerebral edema. Eggs of *T. trichiura* must be differentiated from those of *Capillaria philippinensis*. The eggs of *Capillaria philippinensis* resemble those of *Trichuris trichiura*, but they are broader, more ovoid, and have a striated shell. The lateral plugs of *C. philippinensis* ova do not protrude as in *Trichuris*. The eggs of *C. philippinensis* are also smaller than *Trichuris*, measuring 36 to 45  $\mu\text{m}$  long by 21  $\mu\text{m}$  wide. The adult female worms are 2.5 to 4.3 mm long, and the males are 2.3 to 3.2 mm long.

### 608. *Ascaris lumbricoides* (giant intestinal roundworm)

*Ascaris lumbricoides* is the largest and probably the most prevalent intestinal roundworm infecting humans. The disease is known as ascariasis. The number of people in the world infected with *A. lumbricoides* is presumably second only to the number infected with *E. vermicularis*.

#### Life cycle

The eggs of *Ascaris lumbricoides* are unsegmented when passed and must undergo a period of development in the soil before they are infective. Under favorable conditions, the eggs develop to the infective stage in about 3 weeks. The eggs may remain viable in soil for months or even years. They are very resistant to drying and low temperatures. Once ingested, the digestive juices act on the eggshell, and the larva escapes in the small intestine. The larva migrates through the intestinal mucosa and makes its way to the liver, then the heart, and finally, to the capillaries of the lungs. There it enters an alveolus, where it undergoes one molt and grows to about 2 mm in length. After about 9 days in the alveolus, the third stage larva migrates up the trachea and is swallowed. In the intestines, two more molts occur, and the worms become sexually mature in 8 to 12 weeks from the time the eggs were ingested. Few adult worms live more than 8 months; the maximum life span is less than 18 months.

#### Epidemiology

*Ascaris lumbricoides* is found throughout the world, and the infection is acquired by ingesting embryonated eggs from soil contaminated or fertilized with human feces or uncooked produce contaminated with soil containing infective eggs. However, it is not passed directly from person-to-person or from fresh feces. Heavy infections in children are frequently the result of ingesting soil. Contaminated soil can be carried long distance on feet or footwear and into houses and conveyances; transmission of infection by dust is also possible.

#### Pathology and clinical manifestations

The severity of infection caused by *Ascaris* is attributed to the host's immune response, effects of larval migration, mechanical effects of the adult worms, and nutritional deficiencies due to the presence of the adult worms. Some patients have pulmonary manifestations (pneumonitis, Löffler's syndrome) caused by larval migration and characterized by wheezing, coughing, fever, peripheral eosinophilia, and pulmonary infiltration. Heavy parasite burdens may aggravate nutritional deficiency. Serious complications, sometimes fatal, include bowel obstruction by a bolus (a round mass) of worms, particularly in children; or obstruction of a hollow portion of an organ, such as a bile duct, a pancreatic duct, and the appendix by one or more adult worms. This is usually due to its large size, as stated earlier it is the largest intestinal roundworm infecting humans.

#### Laboratory diagnosis

Identifying eggs in feces or adult worms passed from the anus, mouth, or nose make the diagnosis. Intestinal worms may be visualized by radiological techniques and identifying *Ascaris* larvae, eosinophils, and Charcot-Leyden crystals in the sputum or gastric washings may confirm pulmonary involvement. Larvae have been recovered from sputum specimens, but this is quite rare and not recommended as a routine diagnostic specimen.

#### Eggs

The female may pass either fertile or infertile eggs in the stool. Refer to figure 2-4. The fertilized egg is round or oval (55 to 75  $\mu\text{m}$  long by 35 to 50  $\mu\text{m}$  wide) and contains a single celled ovum. The outer covering of the egg is a mammillated (having nipple-like projections) albuminous coat that is bile-stained golden brown. If the outer mammillated covering is missing from the fertilized egg, the egg shell will be colorless. Unfertilized eggs are generally larger than fertile eggs and are typically more narrow and elliptical, averaging 85 to 90  $\mu\text{m}$  long by 43 to 47  $\mu\text{m}$  wide. Unfertilized eggs may be surrounded by the mammillated albuminous bile-stained coat; however, the coat will appear irregular,

and internally, a globular mass will completely fill the shell. Unfertilized eggs are a common finding, particularly in infections of long standing where there is an absence of males to fertilize the female worm.

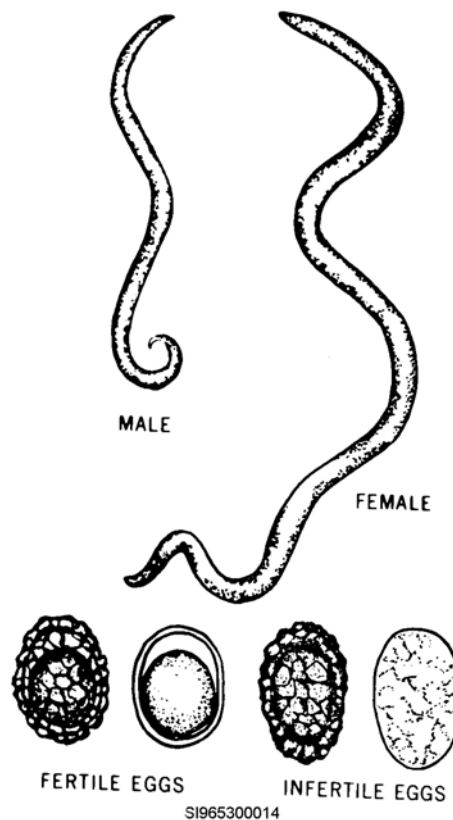


Fig. 2-4. *Ascaris lumbricoides* adult worms and eggs.

### Adult worms

Adult worms can be found in stool specimens and are frequently brought to the laboratory for identification when passed in the stool by the patient. The adults are white or pinkish in color and large. The females measure 20 to 35 cm in length and are about 3 to 6 mm in diameter; the males are roughly 15 to 31 cm long and 2 to 4 mm in diameter. The females produce an average of about 200,000 eggs per day and can produce 27,000,000 eggs in a lifetime. Because of the large volume of eggs passed, you can generally find eggs in simple saline smears of the feces. You do not have to use concentration techniques. Detection of single-worm infections presents difficulties because no eggs, or the peculiar infertile eggs, are found in the stool specimen. You should not rely solely on flotation techniques because infertile eggs are too dense to float with the ordinary techniques. You should always use a direct saline smear in conjunction with any concentration technique.

### Causal Agent

*Ascaris lumbricoides* is the largest nematode (roundworm) parasitizing the human intestine. (Adult females: 20 to 35 cm; adult male: 15 to 30 cm.)

Refer to figure 2-5 for the following references. Adult worms ❶ live in the lumen of the small intestine. A female may produce approximately 200,000 eggs per day, which are passed with the feces ❷. Unfertilized eggs may be ingested but are not infective. Fertile eggs embryonate and become infective after 18 days to several weeks ❸, depending on the environmental conditions (optimum: moist, warm, shaded soil). After infective eggs are swallowed ❹, the larvae hatch ❺, invade the intestinal mucosa, and are carried via the portal, then systemic circulation to the lungs ❻.

The larvae mature further in the lungs (10 to 14 days), penetrate the alveolar walls, ascend the bronchial tree to the throat, and are swallowed **7**. Upon reaching the small intestine, they develop into adult worms **1**. Between 2 and 3 months are required from ingestion of the infective eggs to oviposition by the adult female. Adult worms can live 1 to 2 years.

### ASCARIASIS

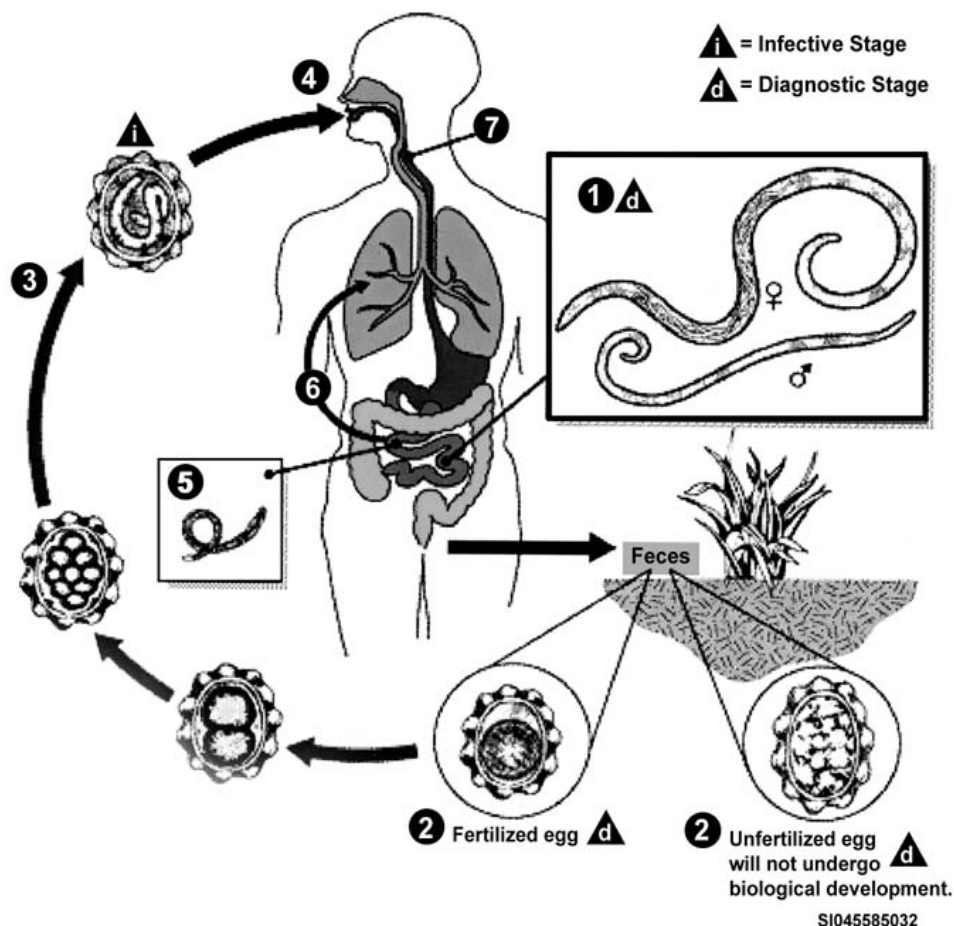


Fig. 2-5. *Ascariasis* life cycle.

### Geographic Distribution

The roundworm is the most common human helminthic infection with worldwide distribution. Highest prevalence is in tropical and subtropical regions, and areas with inadequate sanitation and occurs most in rural areas of the southeastern United States.

### 609. Hookworms

The two species of hookworms of major importance to humans are *Ancylostoma duodenale* (Old World) and *Necator americanus* (New World). They produce a disease known as ankylostomiasis or hookworm infection.

### Life cycle

The life cycles of the hookworms are very similar. The adults attach themselves by their buccal capsules to the mucosa of the small intestine of the host. The female lays a large number of eggs per day (*Necator* produces more than 5,000 and *Ancylostoma* more than 10,000 daily). Passed hookworm eggs are usually in early cleavage and rapidly develop to the first larval stage. When feces containing hookworm eggs are deposited on warm, moist, sandy soil, rhabditiform larvae hatch within 24 to 48

hours. Under favorable conditions, the larvae undergo two molts in about 5 to 8 days. The resulting larvae are third-stage filariform larvae, which are infective. The infective stage filariform larvae of *Necator* infect the human host only by penetrating the skin. The infective larvae of *Ancylostoma* can establish infection after being swallowed or by penetrating the skin. After the larvae penetrate the skin, they enter the blood vessels and are carried to the lungs, where they develop to fourth stage larvae. After about 1 week, they make their way up the pulmonary tree and are swallowed. They attach themselves to the mucosa of the small intestine, where they continue to develop to the adult stage. Then they mate and begin to lay eggs. From the time of skin penetration to egg laying is about 6 weeks. *Ancylostoma* larvae do not undergo any essential development in the lungs, and the filariform infective larvae that are swallowed simply penetrate into the intestinal wall and develop to the fourth stage before they emerge and attach themselves to the intestinal mucosa. Adult worms of both species can live and produce eggs for about 5 to 10 years.

### **Epidemiology**

*A. duodenale* is found principally in southern Europe, northern Africa, China, India, and Japan. *N. americanus* is found in the southern United States, Central and South America, West Indies, Central and South Africa, Southern Asia, South Pacific, and India. Following are the factors controlling the distribution of these parasites:

- Infection of human population.
- Defecation onto soil.
- Acceptable environmental conditions (temperature, rainfall pattern, and open, sandy soil).
- Human contact with the infective larvae in soil.

### **Pathology and clinical manifestations**

Hookworm disease is a common, chronic, parasitic infection with a variety of symptoms. For example, secondary infections rise from entry lesions, pneumonitis, necrosis of intestinal tissue within the adult mouth, and blood loss. The bloodletting activity of the nematode leads to iron deficiency and hypochromic microcytic anemia, the major cause of disability. Children with heavy, long-term infections may have hypoproteinemia and be retarded in mental and physical development. Occasionally, severe acute pulmonary and gastrointestinal reactions follow the exposure to infective larvae. This might occur, for example, in troops confined to foxholes or in other situations permitting intimate exposure to large numbers of infective larvae. Iron deficiency anemia is the classical symptom of hookworm disease. The blood loss is due to sucking by the worm and continuing hemorrhage at the site of attachment, possibly because of anticoagulant secreted by the worm. It is estimated that a *Necator* produces a loss of 0.03 to 0.05 ml of blood per day and an *Ancylostoma*, 0.15 ml of blood per day. *A. duodenale* generally produces a more severe disease than a *N. americanus* does when similar numbers of worms are present. Death is infrequent and can usually be attributed to other infections. Light hookworm infections generally produce few or no clinical effects.

### **Laboratory diagnosis**

Demonstrating hookworm eggs in stool specimens makes diagnosis. You cannot differentiate the species of hookworms on the basis of egg morphology alone. Only on rare occasions will you be required to identify hookworms to species. In the event such identification is required before treatment, set up a “Harada-Mori” culture. After about 1 week check the culture for filariform larvae. Examine the buccal structures of the larvae under high power. The buccal capsule of *Ancylostoma* contains pairs of teeth, while *Necator* contains cutting plates, as shown in figure 2-6.

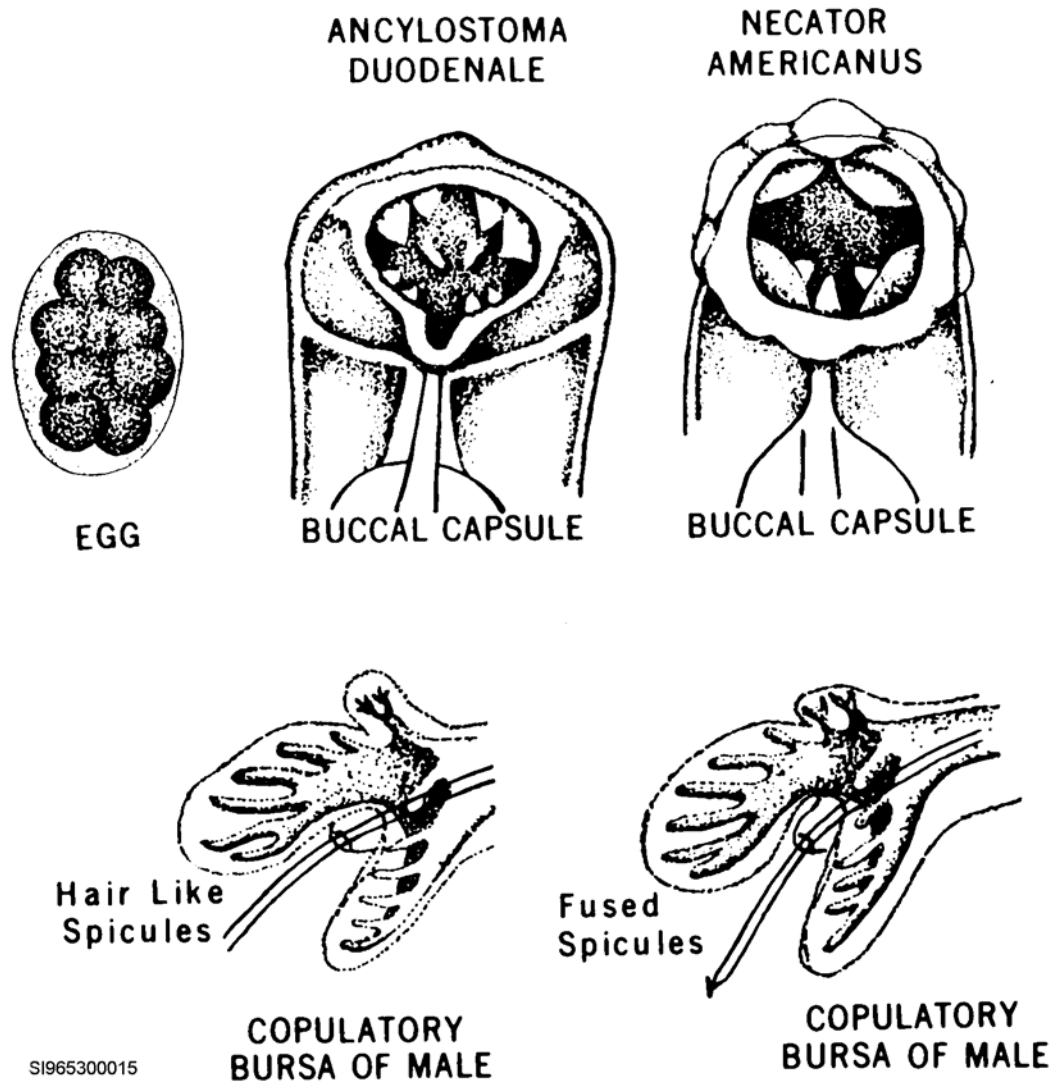


Fig. 2-6. Example of hookworms.

### Adult worms

Having posterior ends modified to form an umbrella-like structure called a bursa further identifies male hookworms. This structure is not present in female worms, which have straight, pointed tails. *Necator* adult worms are 7 to 11 mm long by 0.3 mm wide. *Ancylostoma* worms are somewhat larger; they are 8 to 13 mm long by 0.4 mm wide.

### Eggs

Concentration techniques are not needed to detect the eggs if infection is heavy enough to produce worm disease. On routine fecal examinations, you will recover eggs from patients with only one or two egg-producing worms. The eggs of the hookworms are thin-shelled, partially embryonated, and have a clear space between the developing embryo and the shell. They range from 55 to 75  $\mu\text{m}$  long by 36 to 40  $\mu\text{m}$  wide. To give the physician some idea of the significance of the infection, you should perform a simple egg count. Make a direct saline wet mount from a fresh specimen. Systematically examine the entire smear and count all the eggs. Fewer than five eggs per coverslip indicate light infection that ordinarily does not produce anemia. A count of 25 or more eggs per coverslip indicates heavy infection.

### Causal Agents

Refer to figure 2-7 for the following references. The human hookworms include two nematode (roundworm) species, *Ancylostoma duodenale* and *Necator americanus*. (Adult females: 10 to 13 mm (*A. duodenale*), 9 to 11 mm (*N. americanus*); adult males: 8 to 11 mm (*A. duodenale*), 7 to 9 mm (*N. americanus*). A smaller group of hookworms infecting animals can invade and parasitize humans (*A. ceylanicum*) or can penetrate the human skin (causing cutaneous larva migrans), but do not develop any further (*A. braziliense*, *Uncinaria stenocephala*). Eggs are passed in the stool **1**, and under favorable conditions (moisture, warmth, shade), larvae hatch in 1 to 2 days. The released rhabditiform larvae grow in the feces and/or the soil **2**, and after 5 to 10 days (and two molts) they become become filariform (third-stage) larvae that are infective **3**. These infective larvae can survive 3 to 4 weeks in favorable environmental conditions. On contact with the human host, the larvae penetrate the skin and are carried through the veins to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed **4**. The larvae reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall with resultant blood loss by the host **5**. Most adult worms are eliminated in 1 to 2 years, but longevity records can reach several years. Some *A. duodenale* larvae, following penetration of the host skin, can become dormant (in the intestine or muscle). In addition, infection by *A. duodenale* may probably also occur by the oral and transmammary route. *N. americanus*, however, requires a transpulmonary migration phase.

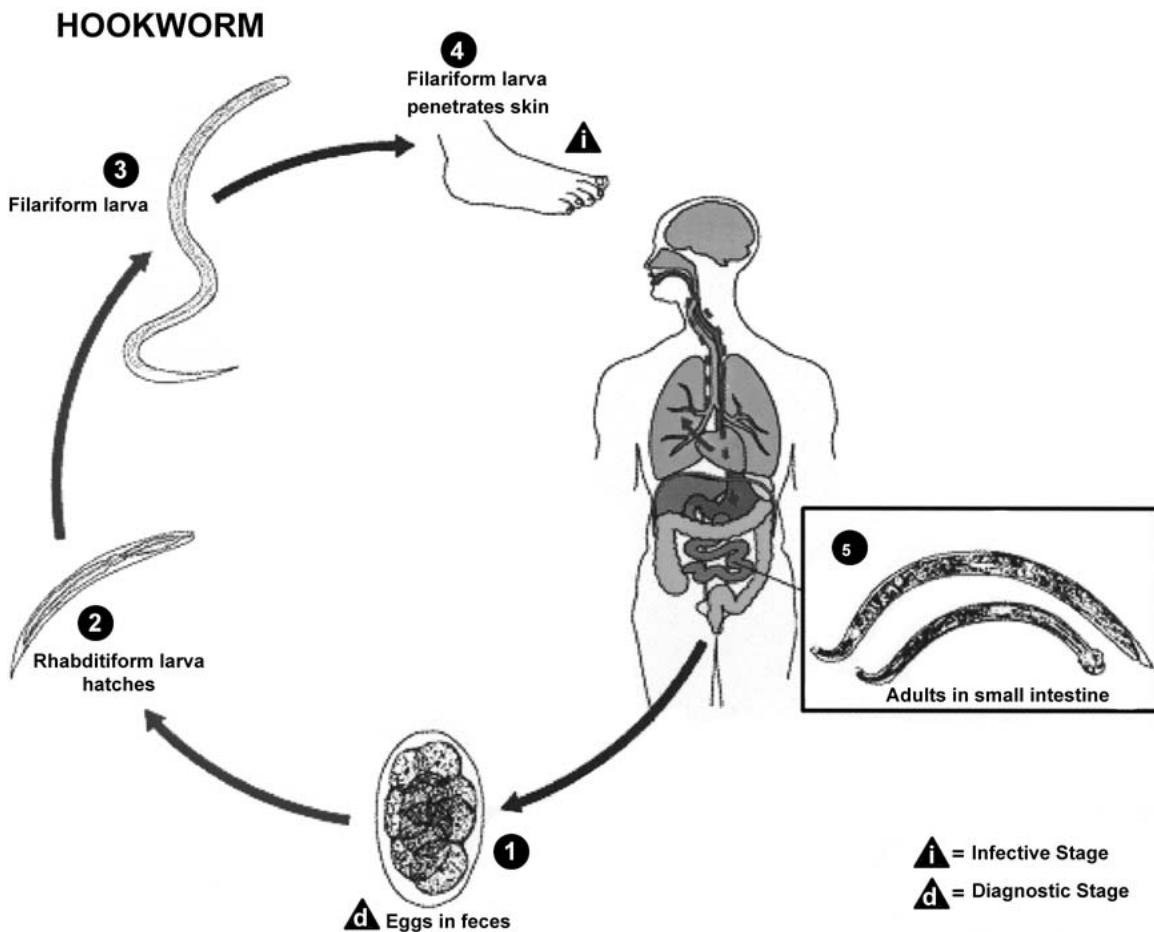


Fig. 2-7. Hookworm life cycle.



### Geographic Distribution

Hookworms are the second most common human helminthic infection (after ascariasis) and are distributed worldwide, mostly in areas with a moist, warm climate. Both *N. americanus* and *A. duodenale* are found in Africa, Asia and the Americas. *Necator americanus* predominates in the Americas and Australia, while only *A. duodenale* is found in the Middle East, North Africa and southern Europe.

### *Trichostrongylus* species overview

*Trichostrongylus* species are nematodes that are commonly found in herbivores, which produces trichostrongylosis. However, some species have been found in humans throughout the world, especially those in rural areas where animals are raised. *Trichostrongylus* species are small worms that live embedded in the mucosa of the small intestine. They are similar to the hookworms, but the adult worms do not have a distinct buccal capsule with special teeth or cutting plates. The eggs pass in the feces and develop in the soil. The third-stage larvae hatch and contaminate plant material (grass, etc.). Humans become infected after ingesting the contaminated plant material (*Trichostrongylus* larvae are *not* capable of invading the skin). Infections with *Trichostrongylus* species are usually not clinically significant unless there are several hundred worms present. If worm burden is high, damage to the intestinal mucosa, hemorrhage, and desquamation (shedding or exfoliation) may occur. They present occasional diagnostic difficulties because their eggs resemble hookworm eggs. *Trichostrongylus* eggs are a little larger than hookworm eggs, measuring 75 to 95  $\mu\text{m}$  long by 40 to 50  $\mu\text{m}$  wide. They have a thin, colorless shell that is tapered slightly at one end. The embryo does not fill the shell so there is a clear space between the embryo and the shell. The adult worms are small and slender, less than 1 cm in length. The males have a prominent bursa. The hatched larvae can be differentiated from those of hookworms and *Strongyloides stercoralis* by the buccal canal, genital primordium, and the larger caudal tip or tail as seen in figure 2-8.

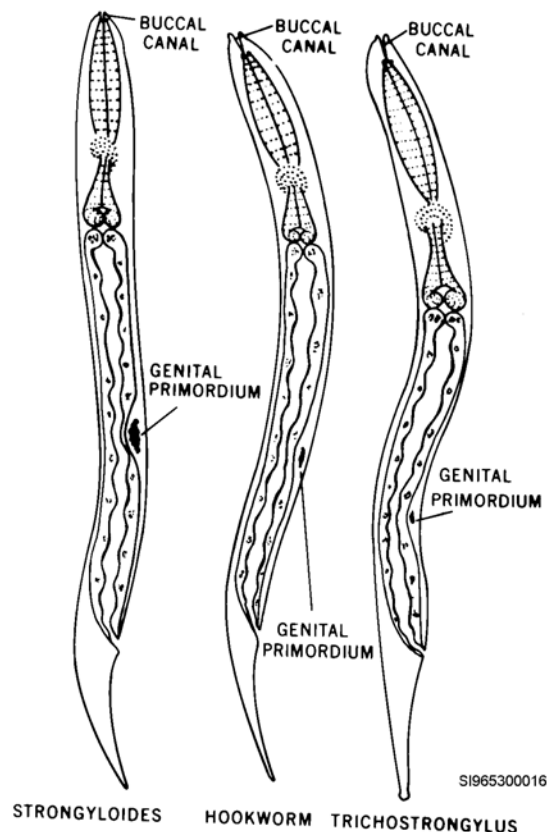


Fig. 2-8. Rhabditoid larvae of *Strongyloides*, Hookworm, and *Trichostrongylus*.

**610. *Strongyloides stercoralis***

*Strongyloides stercoralis* is the causative agent of strongyloidiasis which is generally not as prevalent as the other major intestinal nematode infections.

**Life cycle**

*S. stercoralis* has a rather complicated life cycle. The parasitic adults live in the mucosa of the small intestine. Eggs are passed directly into the mucosa where they embryonate and hatch. Rhabditiform larvae reach the lumen of the intestine and pass in the feces. Once they are passed, they may follow either a direct or an indirect route of development. The indirect route of development occurs when larvae develop into free-living adult male and female worms. This usually happens when the rhabditoid larvae are deposited on warm, moist, shaded soil. The free-living adult females produce eggs, and 2 to 3 days later, free-living rhabditiform larvae hatch. Within 24 hours after the eggs hatch, the rhabditiform larvae develop into infective filariform larvae. Species of *Strongyloides* that parasitize humans have only one completely free-living generation, but some species that parasitize other mammals can have more than one free-living generation. The direct mode of development usually occurs when drier and cooler conditions are prevalent. Under such conditions, the free-living rhabditiform larvae that are deposited on the soil develop directly to infective filariform larvae, thus bypassing the free-living adult stage. The filariform larvae, regardless of mode of development, may live for as long as 2 weeks in the soil. On contact, the infective filariform larvae penetrate the skin and enter the small blood vessels, through which they are carried to the lungs from the heart. In the lungs, they break out of the capillaries into the air spaces (alveoli) and then make their way to the trachea and are swallowed. Once in the intestine, they enter the mucosa of the small intestine, molt twice, and mature in about 2 weeks. There are no males in the parasitic stage; there are only females. The adult female worm is parthenogenetic (produces viable eggs without fertilization by a male worm). In some cases, the rhabditoid larvae transform into infective larvae, penetrate the intestinal wall (autoinfection), and hyperinfection of the host occurs.

**NOTE:** It is important to know that *Strongyloides* infections are frequently latent. The cessation or infrequent passage of larvae in chronic, often asymptomatic, infections may make diagnosis difficult. Multiple specimens should be collected and examined. The disease has been diagnosed in patients who traveled to the endemic area up to 30 years before. If for any reason they become immunocompromised, the result can be a disseminated disease leading to the hyperinfection syndrome and death.

**Epidemiology**

In general, the disease is more common in tropical and subtropical climates where the water table is high. In rainy areas of some countries, such as Brazil, 85 percent of the population may be infected. In the southern United States, surveys found a prevalence of 0.4 to 4 percent. Outside the usual geographic range of infection, Strongyloidiasis may also be endemic or epidemic in crowded settings where personal hygiene is poor, especially in mental hospitals.

**Pathology and clinical manifestations**

The three areas of primary involvement would be the skin, lungs, and the intestinal mucosa. The larvae usually cause little reaction or pruritus and erythema in the skin. With repeated infections, the patient may develop an allergic response. The migration through the lungs may cause pneumonia or Loeffler's syndrome. The tunneling process in the small intestine and the tissue reaction to eggs, worms, and larvae cause epigastric pain, abdominal cramps, and diarrhea. Symptoms may resemble those of a peptic ulcer. Significant eosinophilia is present in about half of the cases. Patients with hyperinfection may have fever and other generalized symptoms. Severe infection, especially if associated with other debilitating conditions (immunosuppression), can lead to death.

### Laboratory diagnosis

Diagnosis is usually made by finding the motile rhabditoid larvae, as shown in figure 2–9, in feces or duodenal contents. However, if the patient is experiencing constipation or autoinfection, filariform stage larvae may be present.

#### *Filariform larvae*

The filariform larvae are approximately 500 to 600  $\mu\text{m}$  by 16  $\mu\text{m}$  wide. When examining filariform larvae, as shown in figure 2–9, note the long slender body shape and relatively long esophagus, which is approximately half of the body's length. The filariform larva of *Strongyloides* can be distinguished from a filariform larva of hookworm because *Strongyloides* has a notched tail; whereas a filariform larva of hookworm has a pointed tail.

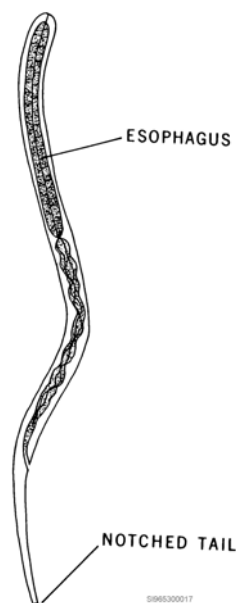


Fig. 2–9 Filariform larvae of *Strongyloides*.

#### *Eggs*

In rare instances in patients with severe infections, the eggs of *Strongyloides* organisms, which are virtually identical in their external appearance to the eggs of the hookworm, may be found in feces. However, the embryo would be much more advanced and contain a nearly completed larval stage worm.

#### *Rhabditiform larvae*

The rhabditoid larvae of *Strongyloides* are 180 to 380  $\mu\text{m}$  long by 14 to 20  $\mu\text{m}$  wide and actively motile. You can see them easily on direct saline smears with the aid of a microscope. In suspected cases where you do not find the larvae on the direct smear, set up a Baermann apparatus, as shown in figure 2–10. The Baermann technique relies on the principle that active larvae will migrate from a fresh fecal specimen that has been placed on the gauze that is in contact with tap water. The larvae will migrate through the gauze, into the water, and settle to the bottom of the funnel where they can be collected and examined. It is important to differentiate rhabditoid larvae of *Strongyloides* from those of hookworms. The points to be especially noted differentiate the two are:

- The buccal canal of *Strongyloides* is very short; whereas that of the hookworm is larger and narrow.
- The genital primordium of *Strongyloides* is much larger than that found in the hookworms.

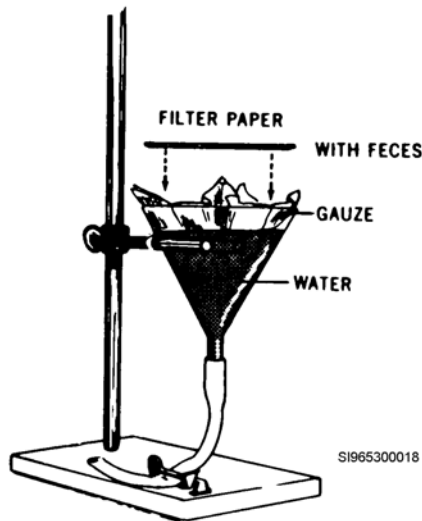


Fig. 2-10. Baermann apparatus.

Other techniques recommended for recovery of larvae include the Entero-Test capsule, Harada-Mori culture, or Petri dish larva culture method.

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### Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

**606. *Enterobius vermicularis* (pinworm)**

1. How do humans become infected with *E. vermicularis*?
2. Once stuck to the perianal folds, how long does *E. vermicularis* ova survive in a humid environment?
3. In what geographic regions is *E. vermicularis* most common?
4. What age group is most frequently infected with *E. vermicularis*?
5. What are the two types of symptoms associated with *E. vermicularis* infections?
6. What conditions may be manifested when female *E. vermicularis* adults migrate to unusual sites, such as the uterus, fallopian tubes, or peritoneal cavity?

7. How can a diagnosis of *E. vermicularis* be made, and how many successive specimens should be taken before enterobiasis is ruled out?
8. How do you describe the eggs of *E. vermicularis*?

**607. *Trichuris trichiura* (whipworm)**

1. How is an infection with *T. trichiura* acquired?
2. How long after being infected with *T. trichiura* do eggs appear in the patient's feces?
3. Infections due to what other organisms often accompany *T. trichiura* infections?
4. How do you describe the symptomology associated with light, moderate, and heavy infections of *T. trichiura*?
5. What will a direct saline smear from a case involving a moderate to heavy infection of *T. trichiura* reveal?
6. How do you describe the diagnosis of *T. trichiura*?
7. How do you describe the eggs of *T. trichiura*?
8. Upon what morphological characteristic can *T. trichiura* and *C. philippinensis* be differentiated?

**608. *Ascaris lumbricoides* (giant intestinal roundworm)**

1. How long can the eggs of *A. lumbricoides* remain viable in the soil?
2. How long, after the ingestion of *A. lumbricoides* eggs, are sexually mature adults found in the intestines?

3. Can *A. lumbricoides* be passed directly from person-to-person?
4. What are some serious complications associated with heavy infections of *A. lumbricoides*?
5. What four types of *A. lumbricoides* eggs can be observed in a fecal sample?
6. How do you describe the appearance of an adult *A. lumbricoides*?
7. Why are concentration techniques not necessary in the laboratory diagnosis of *A. lumbricoides*?

#### **609. Hookworms**

1. What is the infective stage of hookworm larvae?
2. In what two ways can the larvae of *Ancylostoma* establish an infection?
3. What is the life expectancy of adult hookworms?
4. What are the principle factors controlling the distribution of hookworms?
5. What is the major cause of disability experienced by infected patients with hookworms?
6. What is considered the classical symptom of a hookworm infection?
7. Which type of hookworm generally produces a more severe disease?
8. Can the eggs of the different species of hookworm be differentiated?
9. If species identification is required, how is this accomplished?

10. How can you provide the physician with some idea of the significance of infection and what is the procedure?
11. Where are *Trichostrongylus* species commonly found and how are humans infected?

**610. *Strongyloides stercoralis***

1. What route of development occurs when the larvae develop into free-living adult male and female worms?
2. The direct mode of development usually occurs under what climatic conditions?
3. How does the infective filariform larvae enter the small blood vessels?
4. What conditions cause such symptoms as epigastric pain, abdominal cramps, and diarrhea?
5. Diagnosis is usually made by finding the motile rhabditiform larvae in what two body substances?
6. What are the two ways that you can differentiate the rhabditiform larvae of *Strongyloides stercoralis* from those of hookworms?

**2-2. Cestodes (Tapeworms)**

Adult tapeworms are flat, ribbon-like, segmented worms. The individual segments are called *proglottids*. These worms do not have a mouth, gut, or body cavity. They attach themselves to the intestinal mucosa by a scolex (head) that has suckers. The adult worm is separated into the following recognizable regions.

- Scolex.
- Neck, a region of growth that immediately follows the scolex.
- Immature proglottids.
- Mature proglottids.
- Gravid proglottid.

The entire length of the worm that is made up of proglottids is called the *strobila*. The scolex of *Diphyllobothrium latum* is long and spatulate with a long, sucking groove on each side. The other tapeworms that infect humans have knob-like scolices that possess cup-like suckers at each of the

four angles. Some of them also have an interior muscular projection (rostellum) armed with numerous hooks. There is a complete set of male and female organs for each mature proglottid.

*Diphyllobothrium latum* discharges un-embryonated eggs that must reach cool, clear water before further development occurs. The eggs of the other cestodes contain fully developed hexacanth embryos that have three pairs of hooklets. The larval stages of some cestodes can parasitize humans. In such infections, the larvae can be found in almost any tissue of the body. In cases of larval infection by cestodes, such as *Echinococcus*, the infection can be fatal. In some geographic locations, human infections with larval cestodes are quite common. A pathologist usually makes the diagnosis of these larval infections from examination of histological sections of biopsy material. As a means of getting eggs out of the host, some cestodes discharge eggs singularly, some shed one entire gravid proglottid at a time, and some shed groups of gravid proglottids. Recovery and identification of the scolex is necessary for treatment to be considered satisfactory. The five cestodes discussed in this section are *Hymenolepis nana* and *H. diminuta*, *Taenia saginata* and *solium*, and *Diphyllobothrium latum*.

### **611. *Hymenolepis nana* (dwarf tapeworm) and *Hymenolepis diminuta* (rat tapeworm)**

*H. nana* (dwarf tapeworm) and *H. diminuta* are two cestodes that are similar enough to be considered together. *H. nana* produces the disease hymenolepiasis or dwarf tapeworm infection, while *H. diminuta* produces rat tapeworm infection.

#### **Life cycle**

There are differences between these organisms in the areas of life cycle and morphology, and these differences are noted in the text.

#### ***H. nana***

The life cycle for *H. nana* can be either direct or indirect, requiring an intermediate host.

##### *Direct life cycle*

Most human infections are contracted by direct fecal-oral ingestion of the eggs as shown in figure 2-11. Once ingested, the eggs hatch, releasing a hexacanth embryo (oncosphere) into the small intestine. This embryo bores into the mucosal villus (tissue) of the upper small intestine, where it matures into a cysticercoid in 3 to 4 days. The cysticercoid then migrates into the lumen of the small intestine and produces a mature adult. The time lapse between initial infection and the presence of a mature adult worm, measuring 2 to 4 cm and capable of producing eggs, is 2 to 3 weeks. They have a tiny scolex with four suckers and a short rostellum with hooklets. Some *H. nana* eggs are immediately infective when released from the proglottids of the mature adults in the human gut; so autoinfection can occur.

##### *Indirect life cycle*

The indirect life cycle of *H. nana* involves an arthropod, intermediate host. A number of rat and mice fleas, as well as grain beetles, can act as the intermediate host for the dwarf tapeworm. Once *H. nana* eggs are ingested by an insect, they hatch in the insect's intestine, liberating the oncosphere that penetrates into the insect's body cavity and develops into a cysticercoid that is infective to humans, as well as to rodents, when ingested.

#### ***H. diminuta***

This cestode is limited to an indirect life cycle and absolutely requires an arthropod, intermediate host. This is due to the fact that its cysticercoid cannot develop at the higher temperatures encountered in a mammalian, intermediate host. Over 90 species of arthropods may act as the intermediate host for the rat tapeworm. The most common ones are members of the genus *Tribolium* (grain and flour beetles). The adult worms are 20 to 60 cm long with a knob-like scolex, four suckers, and a rostellum *without* hooklets.

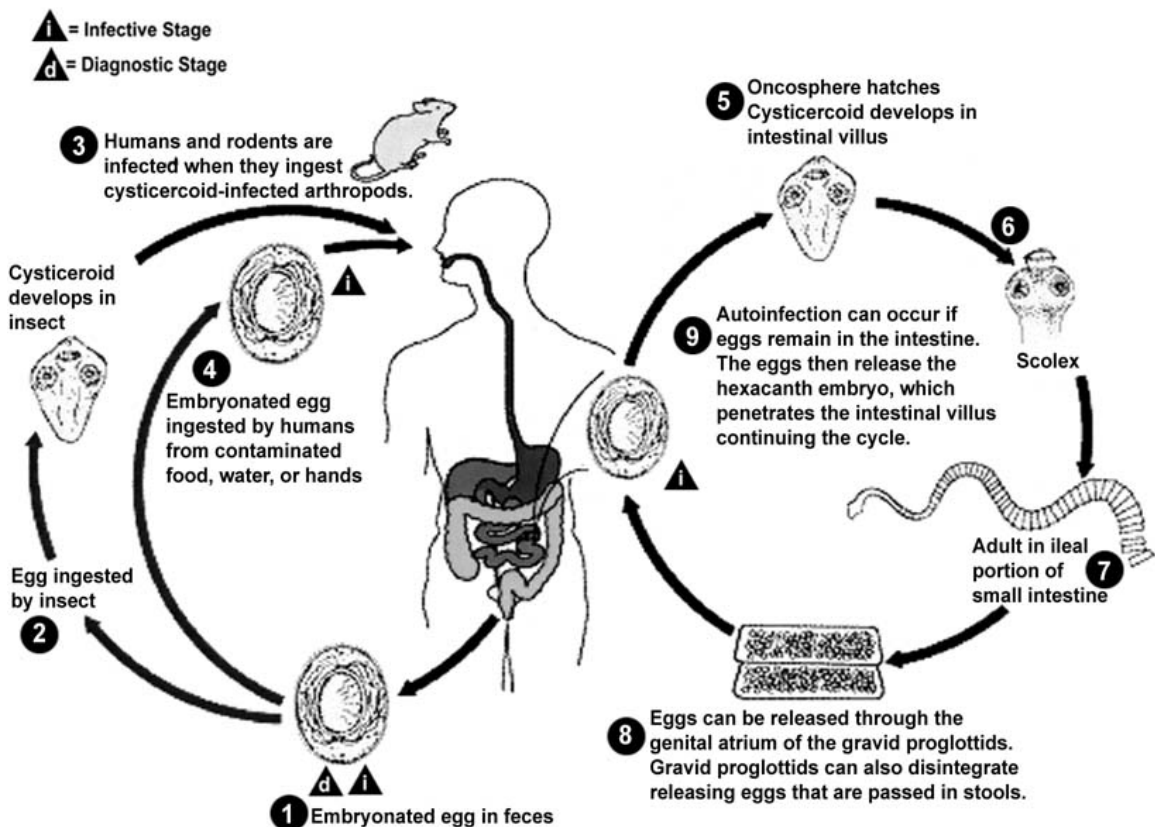


### Causal Agents

Hymenolepiasis is caused by two cestodes (tapeworm) species, *Hymenolepis nana* (the dwarf tapeworm, adults measuring 15 to 40 mm in length) and *Hymenolepis diminuta* (rat tapeworm, adults measuring 20 to 60 cm in length). *Hymenolepis diminuta* is a cestode of rodents infrequently seen in humans and frequently found in rodents.

Refer to figure 2–11 for the following references. Eggs of *Hymenolepis nana* are immediately infective when passed with the stool and cannot survive more than 10 days in the external environment **1**. When eggs are ingested by an arthropod intermediate host **2** (various species of beetles and fleas may serve as intermediate hosts), they develop into cysticeroids, which can infect humans or rodents upon ingestion **3** and develop into adults in the small intestine. A morphologically identical variant, *H. nana* var. *fraterna*, infects rodents and uses arthropods as intermediate hosts. When eggs are ingested **4** (in contaminated food or water or from hands contaminated with feces), the oncospheres contained in the eggs are released. The oncospheres (hexacanth larvae) penetrate the intestinal villus and develop into cysticeroid larvae **5**. Upon rupture of the villus, the cysticeroids return to the intestinal lumen, evaginate their scoleces **6**, attach to the intestinal mucosa and develop into adults that reside in the ileal portion of the small intestine producing gravid proglottids **7**. Eggs are passed in the stool when released from proglottids through its genital atrium or when proglottids disintegrate in the small intestine **8**. An alternate mode of infection consists of internal autoinfection, where the eggs release their hexacanth embryo, which penetrates the villus continuing the infective cycle without passage through the external environment **9**. The life span of adult worms is 4 to 6 weeks, but internal autoinfection allows the infection to persist for years.

### HYMENOLEPIASIS



SI045585044

Fig. 2–11. Life cycle of *Hymenolepis*.

## Epidemiology

*H. nana* is found all over the world, but it is more prevalent in warm, dry climates than in wet, cold ones. The dwarf tapeworm is the most common human tapeworm found in the United States and throughout the world. In the United States, it is found mainly in the southeastern region. It can affect individuals of any age, but it is most prevalent in children. Most infections are acquired person-to-person or from a contaminated play area by direct hand-to-mouth fecal contamination. *H. diminuta* has the same pattern of infection as *H. nana* but is slightly less prevalent. Auto infection does not occur with *H. diminuta*. As we've already mentioned, the mode of transmission of the rat tapeworm is always indirect; therefore, infection is always due to the accidental ingestion of an arthropod, intermediate host. Most infections are linked to the consumption of grains, cereals, etc., that have been contaminated by grain beetles.

## Pathology and clinical manifestations

Light infections of *H. nana* may or may not produce symptoms. There may be diarrhea, vomiting, insomnia, weight loss, and even allergic reactions. Heavy infections almost always produce moderate to profuse diarrhea, nervous disorders, abdominal pain, and, in some cases, extreme apathy. Humans are rarely hosts to more than a few adult rat tapeworms and are rarely symptomatic.

## Laboratory diagnosis

The recognition and differentiation of these two infections are normally based on detection and identification of the characteristic eggs passed in feces. The gravid proglottids of these two parasites usually disintegrate within the intestine and are rarely passed intact in the feces.

### Eggs of *H. nana*

You are expected to be able to identify the characteristic eggs of *H. nana*. Eggs are spherical to ovoid (30 to 47  $\mu\text{m}$  in diameter), gray in color, and contain a hexacanth oncosphere enclosed by an inner and outer eggshell membrane. Polar filaments extend from each end of the egg, between the thin inner and outer egg shells as shown in figure 2-12. These polar filaments are a major diagnostic feature; however, in polyvinyl alcohol fixed specimens, after permanent staining, the very thin outer shell readily collapses. The polar filaments are difficult to detect in these collapsed eggs.

### Eggs of *H. diminuta*

The eggs of *H. diminuta* are similar to those of *H. nana*, as shown in figure 2-12, but with a little study, you should be able to tell them apart. The eggs of *H. diminuta* are rounder and larger, 75 to 85  $\mu\text{m}$  by 60 to 80  $\mu\text{m}$ , and without polar filaments. Because distinguishing the presence of polar filaments is often difficult; the difference in size of the two eggs is frequently used as the major criterion for differentiating the two species.

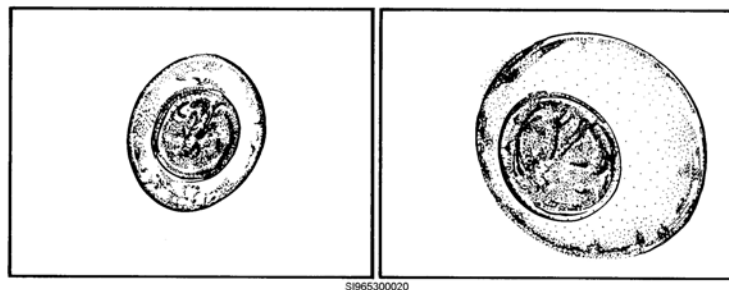


Fig. 2-12. Eggs of *H. nana* on the left and *H. diminuta* on the right.

## 612. *Taenia saginata* (beef tapeworm) and *Taenia solium* (pork tapeworm)

The *Taenia* species infecting humans are *T. saginata* and *T. solium*, which are the causative agents of taeniasis or beef tapeworm infection and pork tape worm infection, respectively.

### Life cycle

The complete life cycle requires a human as the usual definitive host and another animal as an intermediate host. Cattle are the normal intermediate host for *T. saginata* and pigs for *T. solium*. However, because humans can also act as an intermediate host for *T. solium* and develop cysticercosis, a patient harboring an adult *T. solium* is a concern to public health authorities. See figure 2–13 for life cycle schematic. The *cysticercus* of these organisms is ingested in improperly cooked pork or beef. The scolex and neck, which are invaginated into a fluid-filled bladder of the cysticercus, evaginates (turns inside out) under the influence of the bile salts in the small intestines. Then, the scolex attaches to the wall of the small intestine. In the case of *T. saginata*, this is accomplished with four suckers since there are no hooklets (unarmed rostellum) on the scolex. *T. solium* is attached by four suckers plus two rows of hooks located at the anterior end of its scolex. Anchored to the small intestines and bathed in its contents, the organism now enters a period of rapid growth. In 10 to 12 weeks, a mature adult *T. saginata* will be present and measure from 4 to 8 meters, while the more rapidly maturing *T. solium* requires just 5 to 12 weeks to reach 2 to 7 meters. The length of these organisms is composed of a segmented chain of reproductive organs called *proglottids*. In both species, the embryonated eggs contained in gravid (loaded) proglottids is released either within the intestine, if the gravid proglottids disintegrate in the intestine, or after intact proglottids are passed in the fecal mass or actively migrate out of the anus onto the perianal skin (an event almost certain to bring any individual to the hospital). In either situation, the eggs are infective immediately when passed from the body. When a suitable intermediate host ingests the egg, the oncosphere is released in the intermediate host's small intestine, penetrates the intestinal wall, and is carried throughout the body by the lymphatics or circulatory system. Most of the oncospheres localize in the muscles and subcutaneous tissues, where they transform into the larval cysticercus stage within 2 to 3 months. Remember, humans can also act as the intermediate host for *T. solium*; therefore, the eggs of *T. solium* are also infective to humans and cause cysticercosis.

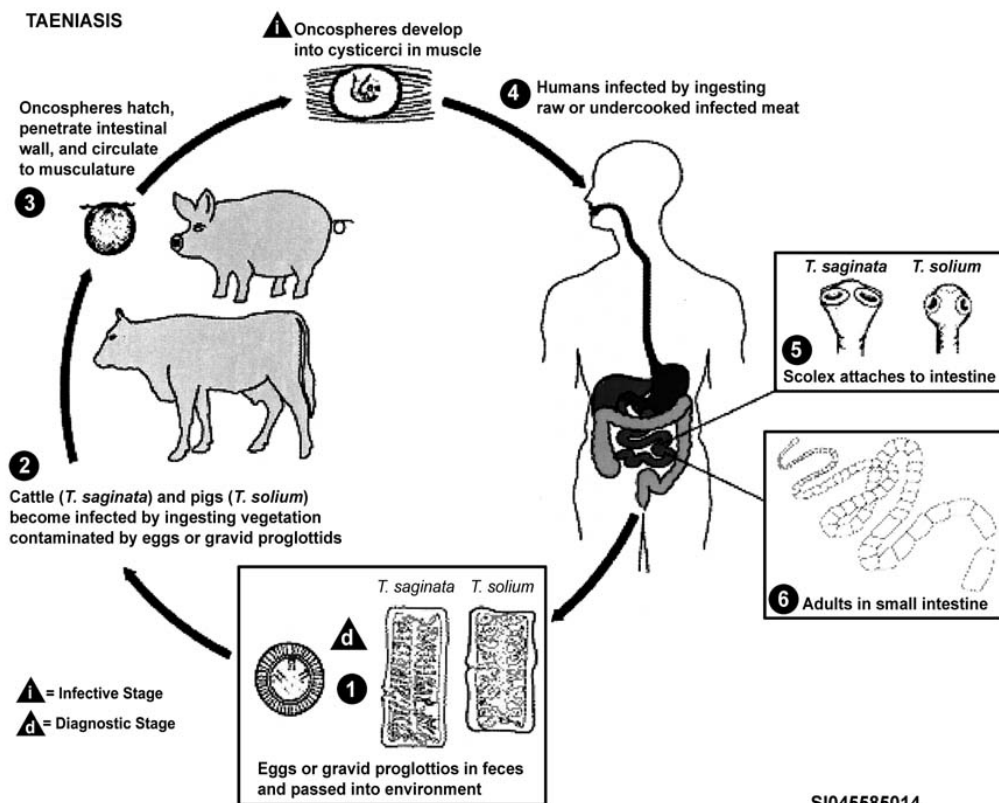


Fig. 2–13. Life cycle of *Taenia*.

### Causal Agents

The cestodes (tapeworms) *Taenia saginata* (beef tapeworm) and *T. solium* (pork tapeworm). *Taenia solium* can also cause cysticercosis.

### Life cycle of *Taenia saginata* and *Taenia solium*

Refer to figure 2-13 for the following references. Humans are the only definitive hosts for *Taenia saginata* and *Taenia solium*. Eggs or gravid proglottids are passed with feces ①; the eggs can survive for days to months in the environment. Cattle (*T. saginata*) and pigs (*T. solium*) become infected by ingesting vegetation contaminated with eggs or gravid proglottids ②. In the animal's intestine, the oncospheres hatch ③, invade the intestinal wall, and migrate to the striated muscles, where they develop into cysticerci. A cysticercus can survive for several years in the animal. Humans become infected by ingesting raw or undercooked infected meat ④. In the human intestine, the cysticercus develops over 2 months into an adult tapeworm, which can survive for years. The adult tapeworms attach to the small intestine by their scolex ⑤ and reside in the small intestine ⑥. Length of adult worms is usually 5 m or less for *T. saginata* (however it may reach up to 25 m) and 2 to 7 m for *T. solium*. The adults produce proglottids which mature, become gravid, detach from the tapeworm, and migrate to the anus or are passed in the stool (approximately 6 per day). *T. saginata* adults usually have 1,000 to 2,000 proglottids, while *T. solium* adults have an average of 1,000 proglottids. The eggs contained in the gravid proglottids are released after the proglottids are passed with the feces. *T. saginata* may produce up to 100,000 and *T. solium* may produce 50,000 eggs per proglottid respectively.

### Geographic Distribution

Both species are worldwide in distribution. *Taenia solium* is more prevalent in poorer communities where humans live in close contact with pigs and eat undercooked pork, and is very rare in Muslim countries.

### Epidemiology

Worldwide distribution is particularly frequent wherever beef or pork is eaten raw or insufficiently cooked or where human waste disposal does not follow good public health principles. Prevalence is highest in parts of Latin America, Africa, Southeast Asia and Eastern Europe. Infection with *T. solium* is rare in the United States and Canada, and exceedingly rare in the United Kingdom and Scandinavia, but it is frequently found in immigrants from endemic areas.

### Pathology and clinical manifestations

Taeniasis is an intestinal infection with the adult stage of large tapeworms; cysticercosis is a tissue infection with the stage of one species, *Taenia solium*. Clinical manifestations of infection with adult worms vary greatly, with some patients experiencing nervousness, diarrhea, vomiting, weight loss, etc. However, most individuals harbor only a single adult and are asymptomatic. The major clinical manifestation and patient complaint is the passage of motile proglottids. Taeniasis is usually a nonfatal infection, but *T. solium* can cause fatal cysticercosis. The larval infection, cysticercosis, is a serious disease that can involve many different organs. Consequences can be grave when larvae localize in the eye, CNS, or heart. In the presence of somatic cysticercosis, epileptiform seizures, signs of intracranial hypertension, or psychiatric disturbances strongly suggest cerebral involvement. Cysticerci in the brain represent the most frequent parasitic infection of the human central nervous system.

### Laboratory diagnosis

Eggs or gravid proglottids passed in the feces, or collected from the perianal fold, can be used for diagnosis of taeniasis. However, a definitive diagnosis differentiating *T. solium* from *T. saginata* cannot be made on the basis of recovered ova alone since taeniid eggs are identical.

### Eggs

The ova of both these organisms are 31 to 43  $\mu\text{m}$  in diameter and have thick, radially striated, dark-brown shells surrounding developed embryos (oncospheres) containing six small hooklets. The only report that can be made based on ova identification is “*Taenia* species found.” Definitive diagnosis must be based on gravid proglottids recovered from a fresh specimen or recovery of the scolex. If the identification is based on the morphology of the scolex, the major criterion is the absence of hooks on the scolex of *T. saginata*.

### Proglottids

When proglottids are used, you can differentiate these organisms by counting the number of main lateral uterine branches observed in a gravid proglottid, as shown in figure 2–14. This is done by squeezing the proglottid between two glass slides and holding it up to a strong light source while using a hand lens. If you are unable to count the uterine branches in this manner, inject the proglottid with India ink to demonstrate the characteristic internal structure. The central tube of the uterus of *T. saginata* has 15 to 20 lateral branches, while there are 7 to 13 lateral branches in the gravid proglottid of *T. solium*.

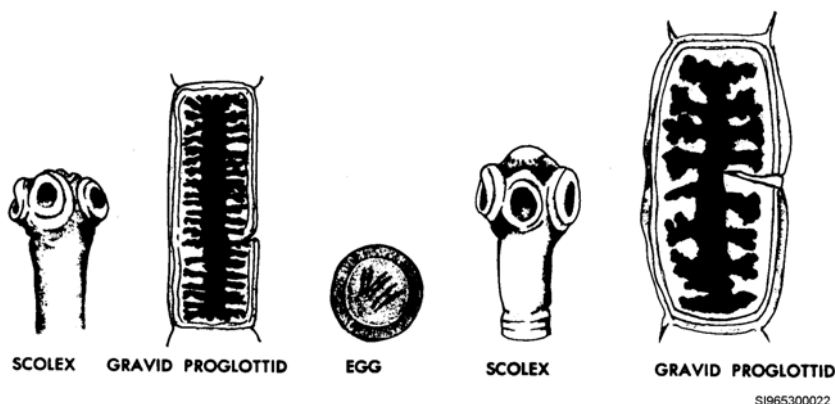


Fig. 2–14. Egg, scolex, and gravid proglottids of *T. saginata* (left) and *T. solium* (right).

### 613. *Diphyllobothrium latum* (fish tapeworm)

*D. latum* is commonly known as the fish tapeworm is the causative agent of diphyllbothriasis or fish tapeworm infection.

#### Life cycle

The eggs are passed in feces and require about 2 weeks in cool, clear water to complete embryonization. When the egg hatches, a coracidium (ciliated six-hooked embryo) escapes through the operculum and swims about. A small crustacean must eat the coracidium within 12 hours. In the crustacean, the coracidium develops into a procercoid larva. A freshwater fish must eat the crustacean infected with the procercoid larva before larval development can be completed. In the fish, the procercoid larva migrates to muscle tissue and develops into a plerocercoid larva (sparganum). When a human or other suitable carnivore ingests raw, poorly cooked, or pickled freshwater fish containing a plerocercoid larva, the larva attaches to the intestine, where it matures and passes eggs, thus completing the cycle. The worm can be up to 10 to 15 meters long and have as many as 3,000 proglottids.

#### Epidemiology

*D. latum* is a common parasite in northern Europe (Scandinavia), Russia, northern Japan, and north America, principally the upper Midwest, Alaska, and Canada. Hosts for this worm include dogs, wolves, bears, and other carnivores (22 species of fish-eating mammals in all), and humans.

### Causal Agents

The cestode *Diphyllobothrium latum* (the fish or broad tapeworm), the largest human tapeworm. Several other *Diphyllobothrium* species have been reported to infect humans, but less frequently; they include *D. pacificum*, *D. cordatum*, *D. ursi*, *D. dendriticum*, *D. lanceolatum*, *D. dalliae*, and *D. yonagoensis*.

Refer to figure 2-15 for the following references. Immature eggs are passed in feces ①. Under appropriate conditions, the eggs mature (approximately 18 to 20 days) ② and yield oncospheres which develop into a coracidia ③. After ingestion by a suitable freshwater crustacean (the copepod first intermediate host) the coracidia develop into proceroid larvae ④. Following ingestion of the copepod by a suitable second intermediate host, typically minnows and other small freshwater fish, the proceroid larvae are released from the crustacean and migrate into the fish flesh where they develop into a plerocercoid larvae (sparganum) ⑤. The plerocercoid larvae are the infective stage for humans. Because humans do not generally eat undercooked minnows and similar small freshwater fish, these do not represent an important source of infection. Nevertheless, these small second intermediate hosts can be eaten by larger predator species, e.g., trout, perch, walleyed pike ⑥. In this case, the sparganum can migrate to the musculature of the larger predator fish and humans can acquire the disease by eating these later intermediate infected host fish raw or undercooked ⑦. After ingestion of the infected fish, the plerocercoid develop into immature adults and then into mature adult tapeworms which will reside in the small intestine. The adults of *D. latum* attach to the intestinal mucosa by means of the two bilateral grooves (bothria) of their scolex ⑧. The adults can reach more than 10 m in length, with more than 3,000 proglottids. Immature eggs are discharged from the proglottids (up to 1,000,000 eggs per day per worm) ⑨ and are passed in the feces ①. Eggs appear in the feces 5 to 6 weeks after infection. In addition to humans, many other mammals can also serve as definitive hosts for *D. latum*.

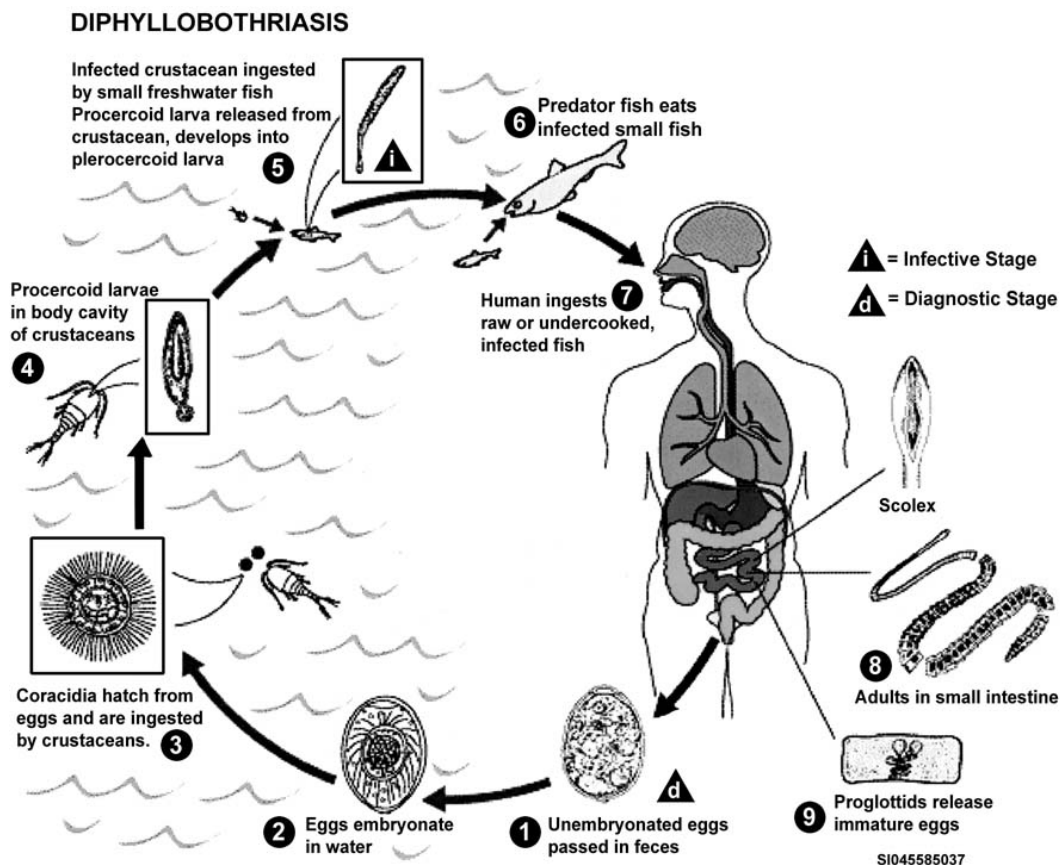


Fig. 2-15. Life cycle of *Diphyllobothrium latum*.

### Geographic Distribution

Diphyllobothriasis occurs in areas where lakes and rivers coexist with human consumption of raw or undercooked freshwater fish. Such areas are found in the Northern Hemisphere (Europe, newly independent states of the former Soviet Union (NIS), North America, Asia), and in Uganda and Chile.

### Pathology and clinical manifestations

Symptoms are commonly trivial or absent. A few patients in whom the worms are attached in the jejunum develop vitamin B<sub>12</sub> deficiency anemia. These individuals are usually over 40 years of age and have diets low in vitamin B<sub>12</sub>. In massive infections, the patient may experience diarrhea, abdominal pain, obstruction of the bile duct or intestine, and toxic symptoms.

### Laboratory diagnosis

Either proglottids or eggs can be detected in the stool. The proglottids are ivory in color, wider than long, and have a rosette-shaped uterus with a central uterine pore (figure 2-16). *D. latum*, unlike other Cestode eggs, are operculated and un-embryonated. The eggs are moderately thick-shelled, yellowish-brown in color, oval, measuring 58 to 75  $\mu\text{m}$  by 44 to 50  $\mu\text{m}$ , and filled with yolk cells, see figure 2-16. The operculum is a cap at the anterior end of the egg through which the free-swimming coracidium may emerge. The operculum may be difficult to detect and, in some cases, light tapping on the wet mount slide may be enough to break open the prominent operculum. A small knob-like protuberance may or may not be present on the egg opposite the operculum (abopercular end).

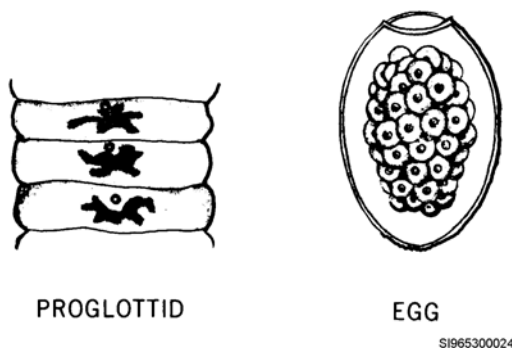


Fig. 2-16. Proglottid and egg of *D. latum*.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 611. *Hymenolepis nana* (dwarf tapeworm) and *Hymenolepis diminuta* (rat tapeworm)

1. How are most human infections of *H. nana* contracted?
2. What is the elapsed time between initial infection by the direct life cycle of *H. nana* and the presence of mature adult worms in the small intestine?
3. Which cestode discussed in this lesson is capable of autoinfection?
4. What does *H. nana* require as an intermediate host in its indirect life cycle?

5. Why does *H. diminuta* absolutely require an intermediate host?
6. What is the most common intermediate host for *H. diminuta*?
7. What is the most common tapeworm infection found in the United States?
8. What are the usual symptoms of a heavy *H. nana* infection?
9. What characteristic of *H. nana* and *H. diminuta* is frequently used as a major criterion for differentiating the two organisms?

**612. *Taenia saginata* (beef tapeworm) and *Taenia solium* (pork tapeworm)**

1. What is the usual definitive host of *T. saginata* and *T. solium*?
2. For which organisms can humans also act as the intermediate host?
3. How do you describe the attachment organs of *T. saginata* and *T. solium*?
4. When are taeniid eggs infective?
5. Usually, what are the major clinical manifestation and the patient complaint associated with taeniasis?
6. Why is the cysticercosis of *T. solium* a serious disease?
7. How can taeniasis be diagnosed?
8. How are taeniid eggs reported?



9. What is the basis of a definitive diagnosis of taeniasis?

**613. *Diphyllobothrium latum* (fish tapeworm)**

1. What are the two intermediate hosts required by *D. latum*?
2. How do humans become infected with *D. latum*?
3. What are the approximate length of the adult worm and the approximate number of proglottids?
4. What type of anemia can a patient with *D. latum* develop?
5. What are the symptoms that accompany a massive *D. latum* infection?
6. How do you describe the proglottids of *D. latum*?
7. How do you describe the ova of *D. latum*?

**2-3. Trematodes (Flukes)**

To understand flukes, you must know about their anatomy and morphology. Take a few moments to study the illustrated life cycles of the organisms discussed in this section.

**614. The schistosomes**

The schistosomes are members of the class *Trematoda*. This class contains nine species of trematodes (flukes), which are important human parasites. Schistosomes are the most important trematodes in humans, in terms of incidence and severity of infection. These include the following schistosomes: two liver flukes, *Fasciola hepatica* and *Clonorchis sinensis*; the giant intestinal fluke, *Fasciolopsis buski*; and the lung fluke, *Paragonimus westermani*. Schistosomes differ in a number of important ways from the rest of the medically important trematodes. For example, the schistosomes, unlike other trematodes, are not hermaphroditic and their eggs are not operculated. They also differ in that they do not require two intermediate hosts or ingestion by a host in order to complete their life cycle. Instead, they require only one intermediate host, a snail, and the infective cercariae can penetrate the exposed skin of a host.

**Life cycle**

Refer to figure 2-17 as you study the life cycle of schistosomes.

## Causal Agents

Schistosomiasis is caused by digenetic blood trematodes. The three main species infecting humans are:

1. *Schistosoma haematobium*.
2. *S. japonicum*.
3. *S. mansoni*.

Two other species, more localized geographically, are *S. mekongi* and *S. intercalatum*. In addition, other species of schistosomes, which parasitize birds and mammals, can cause cercarial dermatitis in humans.

## SCHISTOSOMIASIS

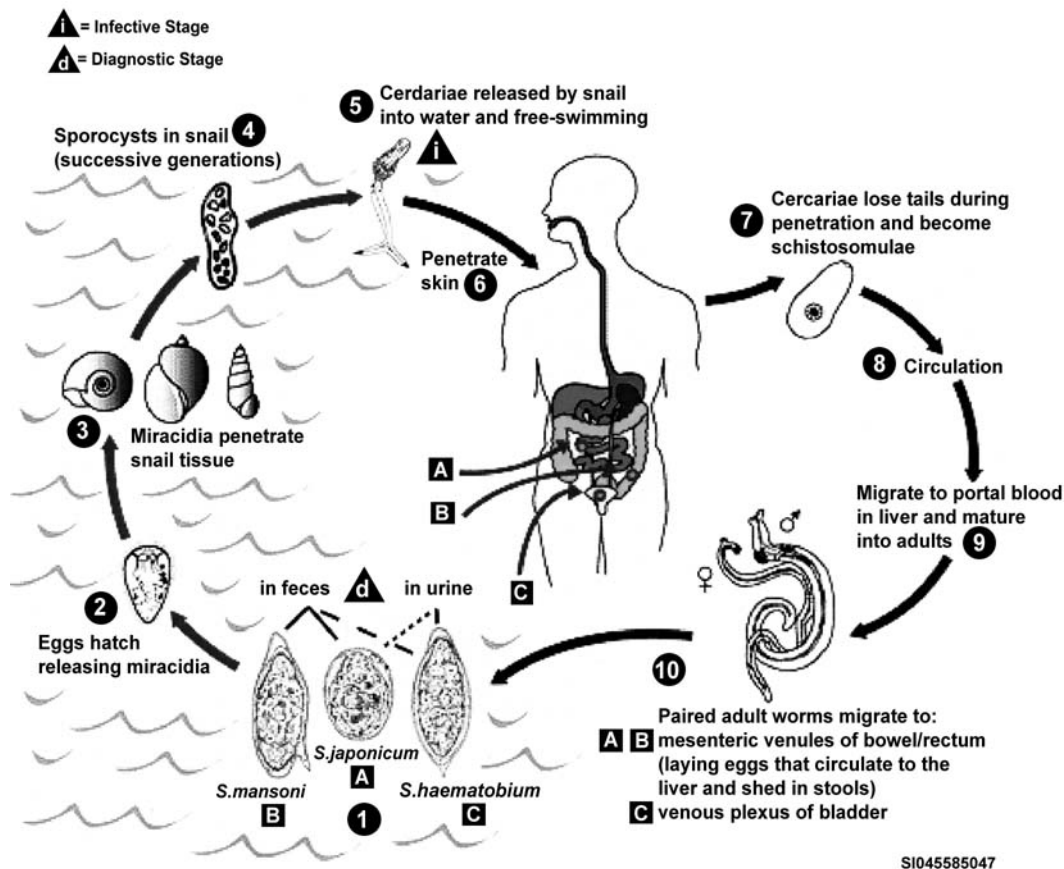


Fig. 2-17. Life cycle of schistosomes.

Refer to figure 2-17 for the following references. Eggs are eliminated with feces or urine (1). Under optimal conditions, the eggs hatch and release miracidia (2), which swim and penetrate specific snail intermediate hosts (3). The stages in the snail include two generations of sporocysts (4) and the production of cercariae (5). Upon release from the snail, the infective cercariae swim, penetrate the skin of the human host (6), and shed their forked tail, becoming schistosomulae (7). The schistosomulae migrate through several tissues and stages to their residence in the veins (8, 9). Adult worms in humans reside in the mesenteric venules in various locations, which at times, seem to be specific for each species (10). For instance, *S. japonicum* is more frequently found in the superior mesenteric veins draining the small intestine (A), and *S. mansoni* occurs more often in the superior mesenteric veins draining the large intestine (B). However, both species can occupy either location, and they are capable of moving between sites, so it is not possible to state unequivocally that one

species only occurs in one location. *S. haematobium* most often occurs in the venous plexus of bladder **C**, but it can also be found in the rectal venules. The females (size 7 to 20 mm; males slightly smaller) deposit eggs in the small venules of the portal and perivesical systems. The eggs are moved progressively toward the lumen of the intestine (*S. mansoni* and *S. japonicum*) and of the bladder and ureters (*S. haematobium*), and are eliminated with feces or urine, respectively. **1** Pathology of *S. mansoni* and *S. japonicum* schistosomiasis includes: Katayama fever, hepatic perisinusoidal egg granulomas, Symmers' pipe stem periportal fibrosis, portal hypertension, and occasional embolic egg granulomas in brain or spinal cord. Pathology of *S. haematobium* schistosomiasis includes: hematuria, scarring, calcification, squamous cell carcinoma, and occasional embolic egg granulomas in brain or spinal cord.

Human contact with water is thus necessary for infection by schistosomes. Various animals, such as dogs, cats, rodents, pigs, horse and goats, serve as reservoirs for *S. japonicum*, and dogs for *S. mekongi*.

### Geographic Distribution

*Schistosoma mansoni* is found in parts of South America and the Caribbean, Africa, and the Middle East; *S. haematobium* in Africa and the Middle East; and *S. japonicum* in the Far East. *Schistosoma mekongi* and *S. intercalatum* are found focally in Southeast Asia and central West Africa, respectively.

### Epidemiology

Five schistosomes are:

1. *S. mansoni*.
2. *S. japonicum*.
3. *S. mekongi*.
4. *S. intercalatum*.
5. *S. haematobium*, infect humans.

#### *S. mansoni*

*S. mansoni* is the most widely distributed of the five medically important schistosomes. It is found in Africa, the Arabian Peninsula, Brazil, Surinam and Venezuela in South America, and in some Caribbean islands. It is also found in several parts of Puerto Rico, and in immigrant populations from Puerto Rico who enter the United States.

#### *S. haematobium*

*S. haematobium* occurs on the African continent, Lebanon, Syria, Iran, and the Arabian peninsula. The Nile River's banks and delta have a particularly high incidence. There are also foci in the Middle East, India, and Madagascar.

#### *S. japonicum*

*S. japonicum* occurs in China, the Philippines, and other countries of southeast Asia. In Japan, human infection has been virtually eliminated.

#### *S. mekongi*

*S. mekongi* is related to *S. japonicum* and occurs only in the Mekong River basin areas of Laos, Cambodia, and Thailand.

#### *S. intercalatum*

*S. intercalatum* occurs in the Democratic Republic of the Congo (formerly Zaire), Gabon, Cameroon, and the Central African Republic.

### Pathology and clinical manifestations

As with most parasites the most important pathological effects of the schistosomes are the complications that arise from chronic infections. A human or animal host can usually tolerate the presence of a few parasites with little or no symptoms or serious long-term effects. However, repeated exposure to the sources of infection can lead to an accumulation of a large number of parasites in the host. For example, in the case of the schistosomes, the daily chore of collecting water from a contaminated source can eventually lead to the acquisition of large numbers of parasites.

#### *S. mansoni*

The adults of *S. mansoni* live in the large intestine and the symptoms exhibited are dependent on the stage of the infection. The prepatent phase of the disease may manifest in many different ways such as fever, skin rash, eosinophilia, diarrhea, and possibly an enlarged liver. In the next phase of development, the parasites lodge in the abdominal mesenteric veins producing pain and dysentery. The characteristic eggs are typically found in the stool during this phase. These symptoms will diminish and chronic phase of the disease will ensue and can persist for many years depending on the parasite burden. The chronic phase will often exhibit granuloma, liver fibrosis, and portal hypertension syndrome.

#### *S. haematobium*

One of the earliest and most common symptoms of *S. haematobium* is hematuria, especially at the end of micturition. In the chronic stages there may be pelvic pain and bladder colic, with increased desire to urinate. Accumulation of eggs in the bladder wall results in hypertrophy of the epithelium, squamous metaplasia, and marked fibrosis, which are responsible for the above symptoms and may lead to urinary obstruction or renal failure. An association between urinary schistosomiasis and squamous cell carcinoma of the urinary bladder has been repeatedly noted, but there is not as yet conclusive proof of a cause-and-effect relationship.

#### *S. japonicum*, *S. mekongi*, and *S. intercalatum*

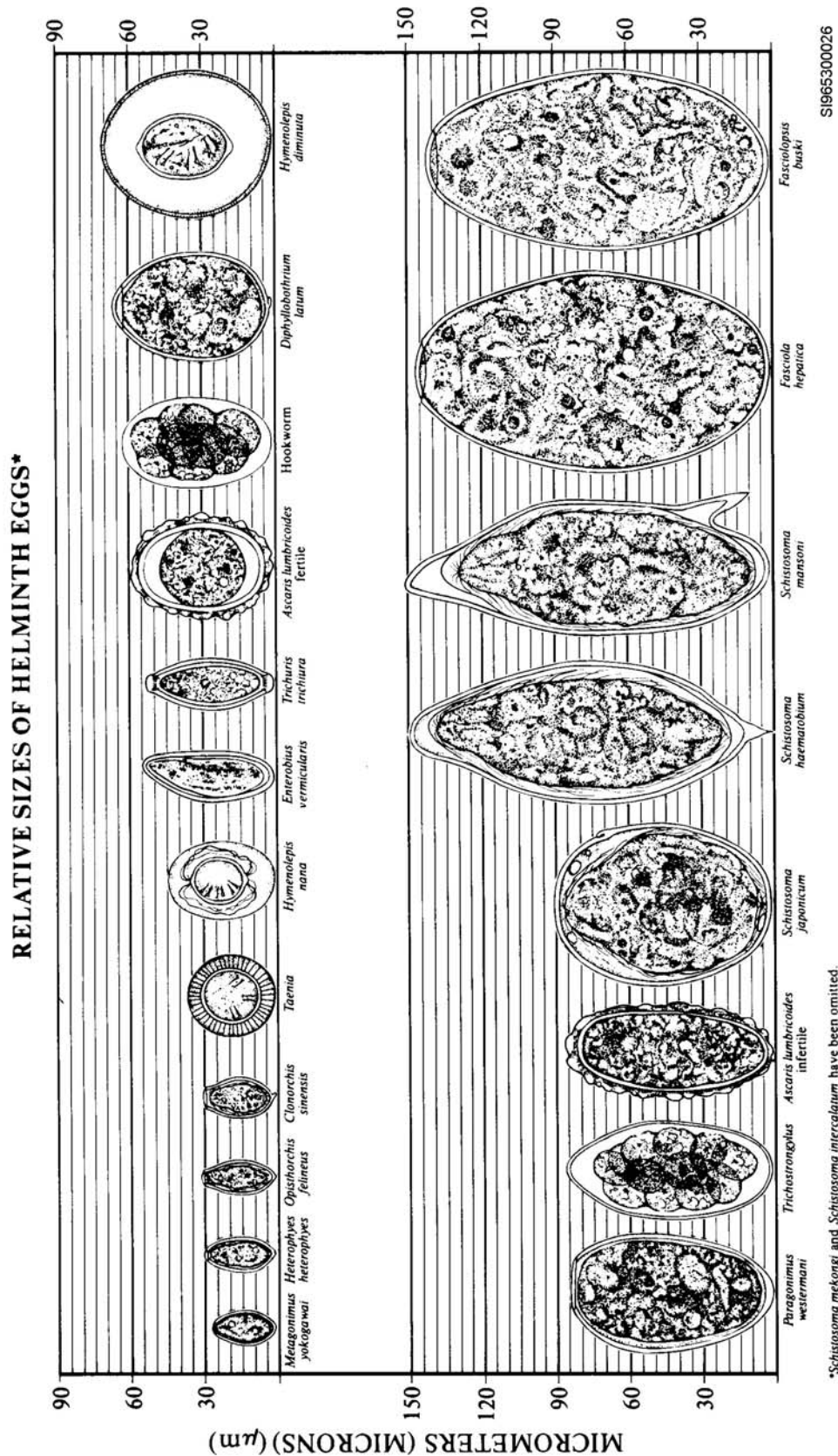
*Schistosoma japonicum's* symptomology, like that of the other schistosomes, is related to the number and location of the parasite in the body. Primarily, it causes intestinal and hepatic symptoms, including diarrhea, abdominal pain, and hepatosplenomegaly. The brain and spinal cord are often involved, and in the Philippines, severe epileptic seizure is often seen. *Schistosoma mekongi* is a newly recognized species that usually remains in the mesenteric veins and causes symptoms similar to those of *S. japonicum*. *S. intercalatum* causes an endemic intestinal disease with abdominal pain, diarrhea, blood and mucus in the stool, and inflammation of rectal walls.

### Laboratory diagnosis

Microscopic identification of the eggs of these parasites is central to a diagnosis of schistosomiasis. In cases in which the ova cannot be recovered, immunological tests may be of help and should be submitted to a Public Health Laboratory or Centers for Disease Control and Prevention.

#### *Eggs of the S. mansoni*

Once visualized, the eggs of the schistosomes can be differentiated from one another on the basis of size and the position of their characteristic spines. The eggs of *Schistosoma mansoni* are 114 to 175  $\mu\text{m}$  long by 45 to 70  $\mu\text{m}$  wide with a prominent lateral spine as shown in figure 2-18. If the spine is tucked under, the coverslip of the wet mount may have to be tapped to move the egg so that it can be better observed.



\*Schistosoma mekongi and Schistosoma intercalatum have been omitted.

Fig. 2-18. Relative sizes of Helminth eggs.

**Eggs of the *S. haematobium***

*S. haematobium* eggs are 112 to 170  $\mu\text{m}$  long by 40 to 70  $\mu\text{m}$  wide and have a very prominent terminal spine.

**Eggs of the *S. japonicum***

*S. japonicum* eggs are smaller than the other species and measure 75 to 100  $\mu\text{m}$  long by 55 to 65  $\mu\text{m}$  wide. They have a minute lateral spine which may or may not be visible.

**Eggs of the *S. mekongi***

*S. mekongi* are smaller than *S. japonicum*, but they are otherwise indistinguishable from the latter. They range from 51 to 78  $\mu\text{m}$  long by 39 to 66  $\mu\text{m}$  wide.

**Eggs of the *S. intercalatum***

*S. intercalatum* are 140 to 240  $\mu\text{m}$  by 50 to 85  $\mu\text{m}$  wide with a very pointed terminal spine.

**615. *Fasciolopsis buski* (giant intestinal fluke)**

This organism is quite prevalent in China, Taiwan, Thailand, Indonesia, India, Vietnam, and other parts of Asia (cases number in the millions), since agricultural practices in these areas are conducive to the life cycle of this organism. It produces the disease fasciolopsiasis.

**Life cycle**

It is typical of other Trematodes in that it is hermaphroditic, requires two intermediate hosts, and has characteristic operculated ova. It differs from the other Trematodes in that its second intermediate host is any of several aquatic plants instead of an aquatic animal. Each adult worm produces approximately 25,000 unembryonated eggs a day, which are passed in the feces of the host (usually pigs or humans). These eggs must be deposited in fresh water. Depending on the water temperature, the eggs will mature and contain miracidia in 3 to 7 weeks. Once the miracidia escapes through the operculum of the ova, they must penetrate a Planorbis snail that acts as the first intermediate host. Each miracidium then transforms through three asexual reproductive stages (a sporocyst and two rediae stages) into the cercariae stage. Once liberated from the snail, the cercariae encyst on aquatic vegetation (water chestnuts, water caltrops, bamboo shoots, and other edible plants) and become the infective metacercariae. See figure 2-19 for an example of life cycle. Human infection results from eating these plants uncooked. The metacercariae excyst and attach to the duodenal or jejunal mucosa. In about 3 months, they develop into an adult worm.

**Causal Agent**

The trematode *Fasciolopsis buski*, the largest intestinal fluke of humans. Immature eggs are discharged into the intestine and stool ①. Eggs become embryonated in water ②, eggs release miracidia ③, which invade a suitable snail intermediate host ④. In the snail the parasites undergo several developmental stages (sporocysts ④a, rediae ④b, and cercariae ④c). The cercariae are released from the snail ⑤ and encyst as metacercariae on aquatic plants ⑥. The mammalian hosts become infected by ingesting metacercariae on the aquatic plants. After ingestion, the metacercariae excyst in the duodenum ⑦ and attach to the intestinal wall. There they develop into adult flukes (20 to 75 mm by 8 to 20 mm) in approximately 3 months, attached to the intestinal wall of the mammalian hosts (humans and pigs) ⑧. The adults have a life span of about one year.

**Geographic Distribution**

Asia and the Indian subcontinent, especially in areas where humans raise pigs and consume freshwater plants.

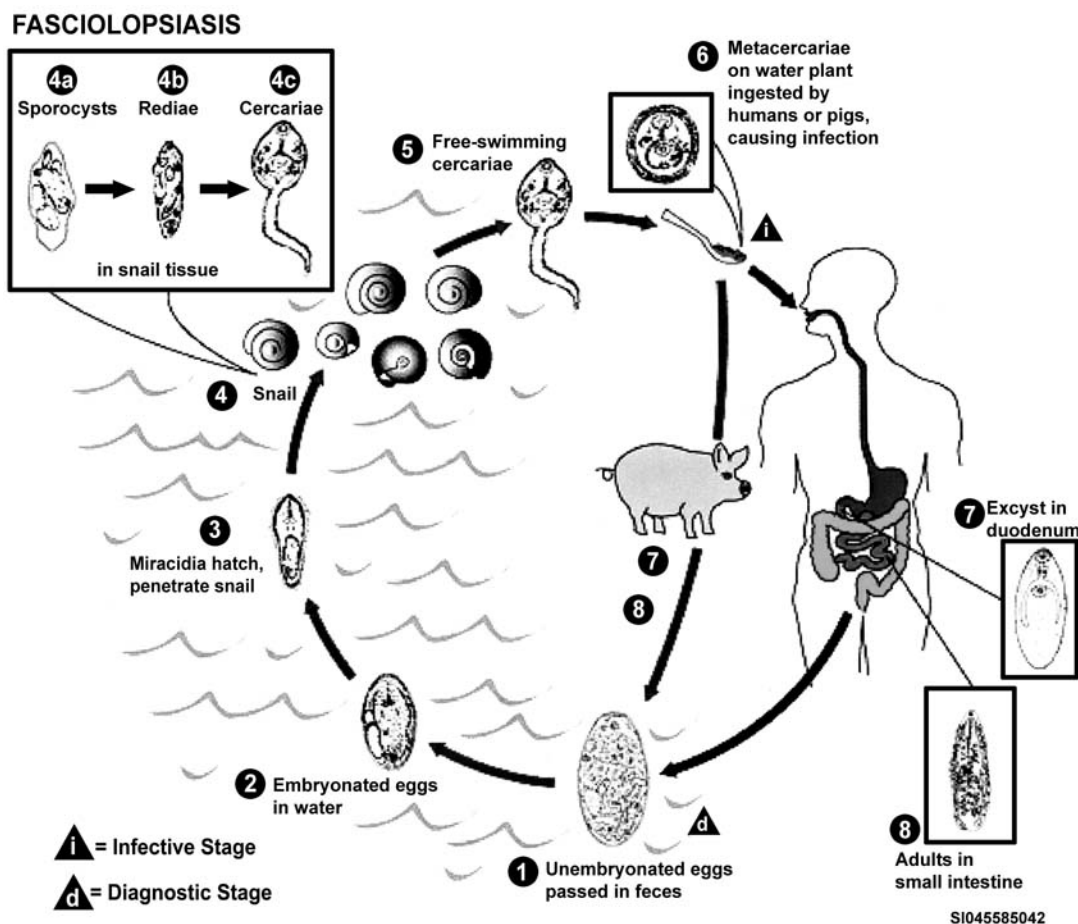


Fig. 2-19. Life cycle of *Fasciolopsis buski*.

### Epidemiology

This organism is particularly widespread in the Far East where human and animal feces are frequently used as a source of fertilizer for crops grown in water for human consumption. This practice regularly introduces large numbers of ova into water supplies that are, also, home to suitable intermediate hosts. Thus, all the elements necessary for the continuation of the parasites' life cycle are available in abundance. The result of such practices is tens of millions of cases of *F. buski* worldwide, especially in rural areas of Bangladesh, Taiwan, Thailand, and Vietnam.

### Pathology and clinical manifestations

*F. buski* is the largest and most pathogenic of the human intestinal flukes. The intestinal tracts of the majority of patients with this parasitic infection contain only a few adult flukes, and they are, for the most part, asymptomatic. Patients with heavy infections, however, experience a variety of symptoms. These symptoms can be characterized as traumatic, obstructive, and toxic.

#### Traumatic

Most common are vague abdominal symptoms similar to those seen in patients with duodenal ulcers who experience pain and complain of feeling constantly hungry. Other symptoms include alternating bouts of diarrhea and constipation as well as vomiting. These symptoms are due to inflammation, ulceration, and hemorrhage at the attachment site. This inflammation provokes excess mucous secretion, which is a typical symptom of infection.



### Obstruction

In heavy infections, the worms may be found in the stomach and intestinal tract. Therefore, they can block the passage of food and interfere with normal digestive juice secretion.

### Toxic or allergic reactions

Verminous intoxication is another aspect of a heavy parasitic infection with this organism and is especially prevalent in children. It is a profound toxic or allergic reaction to the absorption of the worm's metabolites. The patient exhibits edema and ascites (effusion and accumulation of serous fluid in the abdominal cavity). Heavy infections can also cause death.

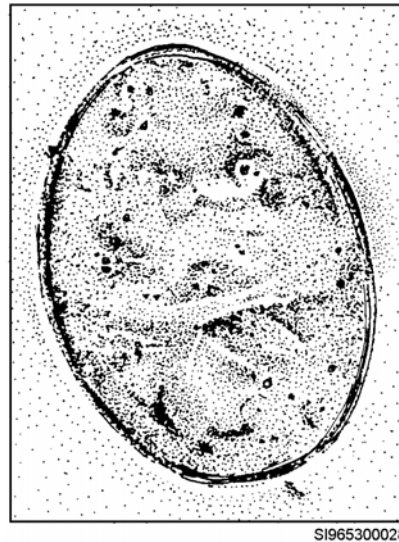


Fig. 2-20. Egg of *Fasciolopsis buski*.

### Laboratory diagnosis

Diagnosis is made by finding the large flukes measuring up to 75 mm long by 20 mm wide, or the characteristic eggs, as shown in figure 2-20, in feces. Occasionally, the adult flukes are seen and identified in patients' vomitus. The most common method of diagnosis is the microscopic identification of the 130 to 140  $\mu$ m long by 80 to 85  $\mu$ m wide unembryonated operculated eggs in the patient's feces. The eggs are usually easily seen in a direct smear. However, the fact that they cannot be differentiated from the eggs of *Fasciola hepatica* makes it common practice to report both of these organisms out as "*Fasciola/Fasciolopsis* seen."

### 616. *Fasciola hepatica* (sheep liver fluke)

Unlike the other Trematodes already discussed, humans are an accidental host of *F. hepatica*, its natural primary host being sheep. Unlike the other parasitic infections we've discussed, finding the eggs of *F. hepatica* in the patient's feces doesn't necessarily mean that the patient has a parasitic infection, since a false fascioliasis can occur if the patient has consumed liver from an infected animal.

### Life cycle

Refer to figure 2-21 as you study the life cycle of *F. hepatica*.

### Causal Agent

Immature eggs are discharged in the biliary ducts and in the stool ①. Eggs become embryonated in water ②, eggs release miracidia ③, which invade a suitable snail intermediate host ④, including many species of the genus *Lymnae*. In the snail the parasites undergo several developmental stages (sporocysts ④a, rediae ④b, and cercariae ④c). The cercariae are released from the snail ⑤ and encyst



as metacercariae on aquatic vegetation or other surfaces. Mammals acquire the infection by eating vegetation containing metacercariae. Humans can become infected by ingesting metacercariae-containing freshwater plants, especially watercress **6**. After ingestion, the metacercariae excyst in the duodenum **7** and migrate through the intestinal wall, the peritoneal cavity, and the liver parenchyma into the biliary ducts, where they develop into adults **8**. In humans, maturation from metacercariae into adult flukes takes approximately 3 to 4 months. The adult flukes (*Fasciola hepatica*: up to 30 mm by 13 mm; *F. gigantica*: up to 75 mm) reside in the large biliary ducts of the mammalian host. *Fasciola hepatica* infect various animal species, mostly herbivores.

### FASCIOLIASIS

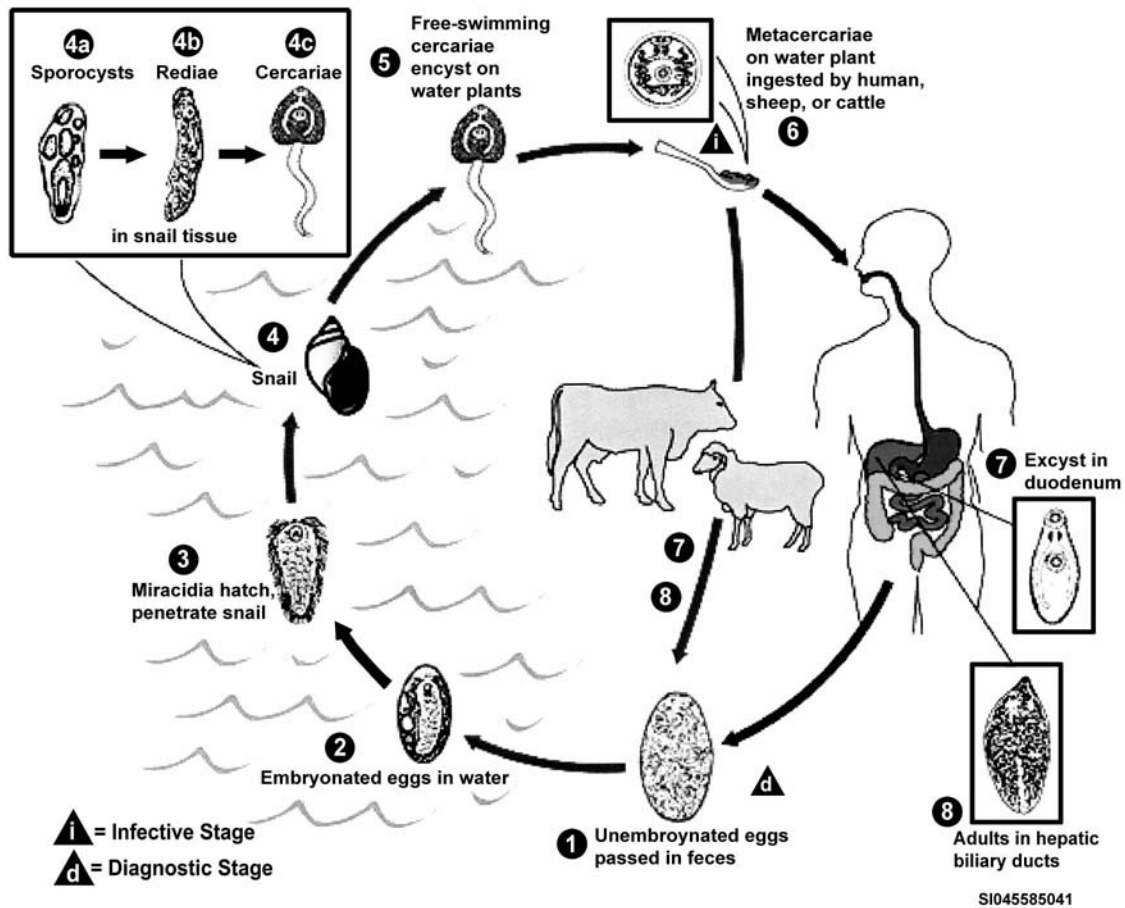


Fig. 2-21. *Fasciola hepatica* life cycle.

### Geographic Distribution

Fascioliasis occurs worldwide. Human infections with *F. hepatica* are found in areas where sheep and cattle are raised, and where humans consume raw watercress, including Europe, the Middle East, and Asia. Infections with *F. gigantica* have been reported, more rarely, in Asia, Africa, and Hawaii.

### Epidemiology

Human infection is common in parts of Europe, northern Africa, Cuba, South America, and other sheep or cattle raising locales worldwide. Very few cases of human fascioliasis have been reported in the United States, even though the infection is common in sheep in the southern and western parts of the country.

**Pathology and clinical manifestations**

Headache, chills, fever, and right upper quadrant pain with hepatomegaly manifest human fascioliasis. Jaundice, diarrhea, and anemia may occur in severe infections, and hepatic cirrhosis of the biliary type is a late complication.

**Laboratory diagnosis**

Microscopic identification of the large 130 to 150  $\mu\text{m}$  long by 63 to 90  $\mu\text{m}$  wide, thin-walled operculated eggs in the patient's feces is typically the manner in which a definitive diagnosis is made. In cases where the spurious presence of eggs is suspected, the patient should be instructed to abstain from eating liver for 3 days, and then a repeat stool examination should be performed. Adult worms are fleshy looking and measure up to 30 mm long by 13 mm wide.

**617. *Clonorchis sinensis* (oriental liver fluke) and *Opisthorchis* species**

The trematodes *Clonorchis sinensis* and *Opisthorchis* species have identical life cycles and there are only slight differences in the morphology of the various stages of the parasites.

**Life cycle**

Human infections with these parasites are acquired by ingestion of raw or improperly cooked fish containing cyst-like larval stage of the parasite (metacercariae). During digestion, larvae are freed from cysts and migrate via the common bile duct to biliary radicles (the smallest branches of the biliary tree). Eggs deposited at these sites are carried with the bile into the intestines and leave the body in the feces. These eggs contain a fully embryonated miracidium that must be ingested by an appropriate species of fresh water snail before they can undergo a series of asexual reproductive stages (sporocysts and rediae). This portion of the life cycle is complete when large numbers of infective cercariae are released into the water. The cercariae then seek out an acceptable species of fresh water fish as a second intermediate host. Over 40 species of fish can act as the second intermediate host. Upon contact with a second intermediate host, the cercariae penetrate the host fish and encyst, usually in muscle. The complete life cycle, as shown in figure 2-22, from person-to-snail to fish-to-person, requires at least 3 months.

**Casual Agent**

The trematode *Clonorchis sinensis* (Chinese or oriental liver fluke). Embryonated eggs are discharged in the biliary ducts and in the stool ①. Eggs are ingested by a suitable snail intermediate host ②; there are more than 100 species of snails that can serve as intermediate hosts. Each egg releases a miracidia ②a, which go through several developmental stages (sporocysts ②b, rediae ②c, and cercariae ②d). The cercariae are released from the snail and after a short period of free-swimming time in water, they come in contact and penetrate the flesh of freshwater fish, where they encyst as metacercariae ③. Infection of humans occurs by ingestion of undercooked, salted, pickled, or smoked freshwater fish ④. After ingestion, the metacercariae excyst in the duodenum ⑤ and ascend the biliary tract through the ampulla of Vater ⑥. Maturation takes approximately 1 month. The adult flukes (measuring 10 to 25 mm by 3 to 5 mm) reside in small and medium sized biliary ducts. In addition to humans, carnivorous animals can serve as reservoir hosts.

**Geographic Distribution**

Endemic areas are in Asia including Korea, China, Taiwan, and Vietnam. Clonorchiasis has been reported in nonendemic areas (including the United States). In such cases, the infection is found in Asian immigrants, or following ingestion of imported, undercooked or pickled freshwater fish containing metacercariae.

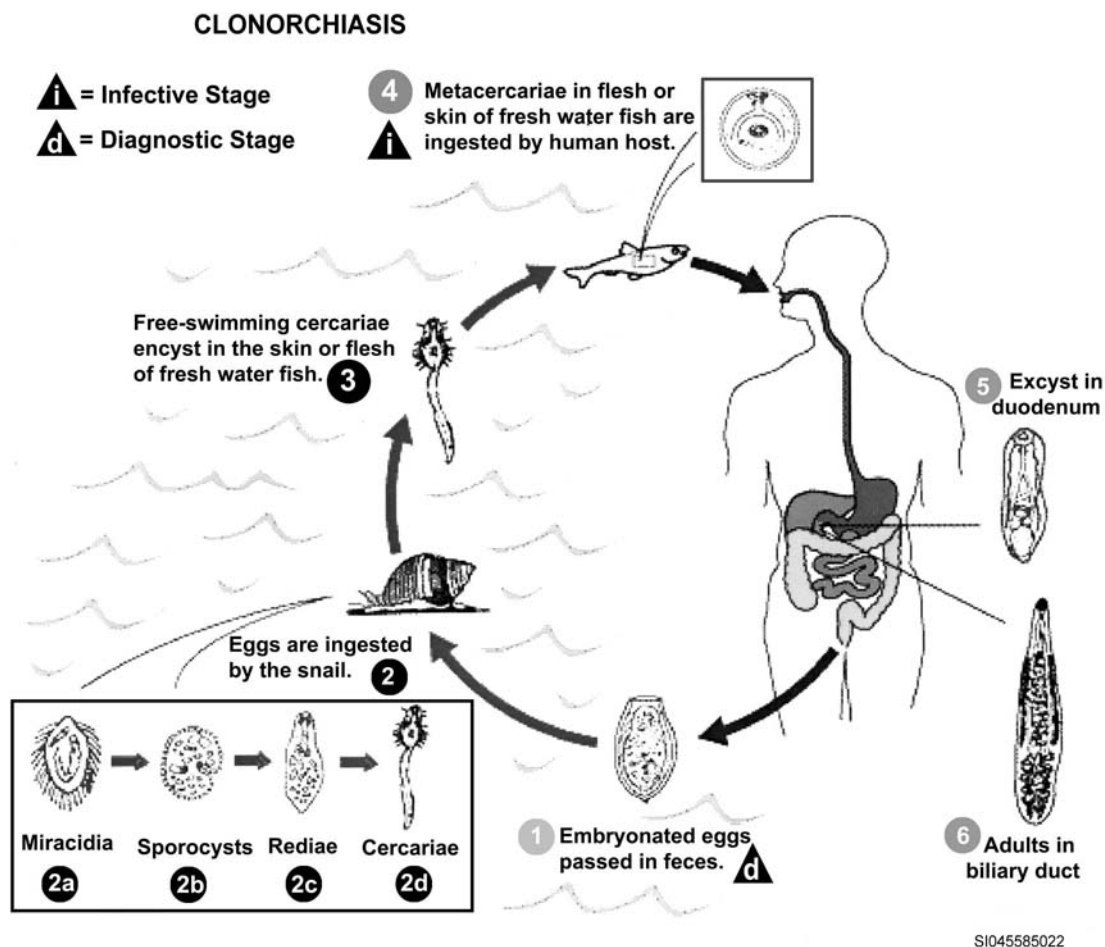


Fig. 2-22. Life cycle of *Clonorchis sinensis*.

### Epidemiology

Clonorchiasis and opisthorchiasis are considered to be zoonotic parasitic infections (due to animal parasites), since the usual primary hosts are domestic and wild fish eating animals. However, human cases are very common in endemic areas (Orient) due to centuries-old eating habits and the use of night soil in the commercial production of fish. Both rich and poor are afflicted; the former are infected as they dine on beautifully cut and arranged slices of raw fish, and the latter since they depend on fish as their only source of protein and cooking fuel is a luxury that they cannot afford. In 1947, it was estimated that approximately 19 million people were infected with *C. sinensis*, and the number is probably higher today. *C. sinensis* is highly endemic in China, Japan, Taiwan, South Korea, and Vietnam, principally in the Red River delta.

### Pathology and clinical manifestations

The basic pathologic change in these infections is a parasite-induced inflammation of the lining of the bile ducts and hyperplasia (increase in the number of normal cells in a tissue) of the endothelial lining the ducts. This change is due to the body's response to the chronic defoliation of the biliary epithelium leading to the gradual thickening and occlusion of the bile ducts. Fortunately, most infected individuals are asymptomatic, but patients with heavy worm burdens of long-standing duration do face serious consequences. At the very least, these patients have colicky pain, diarrhea, and ascites. However, the walls of the bile ducts may develop pockets that eventually perforate, allowing ova to infiltrate liver tissue. Liver function is ultimately affected, resulting in hepatitis, cirrhosis, and liver malignancies.

### Laboratory diagnosis

Since egg production is relatively low (2,500 per day) in comparison to other parasitic organisms, a concentration procedure is strongly recommended. Eggs measure 23 to 35  $\mu\text{m}$  long by 12 to 20  $\mu\text{m}$  wide. Eggs of *Clonorchis*/*Opisthorchis* often have a hook on the opercular end and are narrower at the end with the flattened operculum. *Heterophyes*/*Metagonimus* lack the hook and are wider and more round at the operculated end. Visualization of what appears to be the ova of *C. sinensis*, as shown in figure 2-23, or *Opisthorchis* species is not a definitive means of diagnosis since the ova of these organisms, as well as two additional genera, *Heterophyes* and *Metagonimus*, are almost identical and are difficult to differentiate. Because of the differentiation difficulty of these organisms ova, the generic diagnosis of *Clonorchis*/*Opisthorchis*/*Heterophyes*/*Metagonimus* species may be reported by some laboratories.

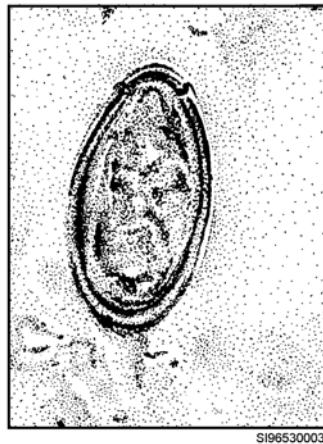


Fig. 2-23. Egg of *Clonorchis sinensis*.

### 618. *Paragonimus westermani* (oriental lung fluke)

*Paragonimus westermani* is the causative agent of paragonimiasis or oriental lung fluke infection.

#### Life cycle

Adult *P. westermani* (lung fluke) usually live in the lungs in encapsulated pairs. The capsule is of host origin as a response to the inflammation caused by their presence. It is common to find the adult organisms in various ectopic sites such as the lymph nodes, brain, and peritoneal cavity. The eggs escape from the lungs via the air passages. They are swept along the bronchi by the cilia of the epithelium. They are then coughed up and exit the body in expectorated sputum or swallowed, exiting with the feces. Once they reach water, the eggs require 16 days to several weeks for the complete development of the miracidium, which then hatch and seek out an appropriate snail host. Upon entering the snail, the miracidium forms a sporocyst that produces rediae, which in turn develop many cercariae, as shown in figure 2-24. These cercariae then attack crabs or crayfish of at least 11 species and encyst in their viscera and muscles. Eating the raw or improperly cooked crabs or crayfish infects humans. The encysted organisms then excyst in the duodenum, pierce its wall, and embed into the abdominal wall. Several days later, they reenter the body cavity, penetrate the diaphragm and pleura, and enter the bronchioles of the lungs. They mature in 8 to 12 weeks.

#### Causal Agent

More than 30 species of trematodes (flukes) of the genus *Paragonimus* have been reported which infect animals and humans. Among the more than 10 species reported to infect humans, the most common is *P. westermani*, the oriental lung fluke.

The eggs are excreted unembryonated in the sputum, or alternately they are swallowed and passed with stool ❶. In the external environment, the eggs become embryonated ❷, and miracidia hatch

and seek the first intermediate host, a snail, and penetrate its soft tissues <sup>3</sup>. Miracidia go through several developmental stages inside the snail <sup>4</sup>: sporocysts <sup>4a</sup>, rediae <sup>4b</sup>, with the latter giving rise to many cercariae <sup>4c</sup>, which emerge from the snail. The cercariae invade the second intermediate host, a crustacean such as a crab or crayfish, where they encyst and become metacercariae. This is the infective stage for the mammalian host <sup>5</sup>. Human infection with *P. westermani* occurs by eating inadequately cooked or pickled crab or crayfish that harbor metacercariae of the parasite <sup>6</sup>. The metacercariae excyst in the duodenum <sup>7</sup>, penetrate through the intestinal wall into the peritoneal cavity, then through the abdominal wall and diaphragm into the lungs, where they become encapsulated and develop into adults <sup>8</sup> (7.5 to 12 mm by 4 to 6 mm). The worms can also reach other organs and tissues, such as the brain and striated muscles, respectively. However, when this takes place completion of the life cycles is not achieved, because the eggs laid cannot exit these sites. Time from infection to oviposition is 65 to 90 days. Infections may persist for 20 years in humans. Animals such as pigs, dogs, and a variety of feline species can also harbor *P. westermani*.

### PARAGONIMIASIS

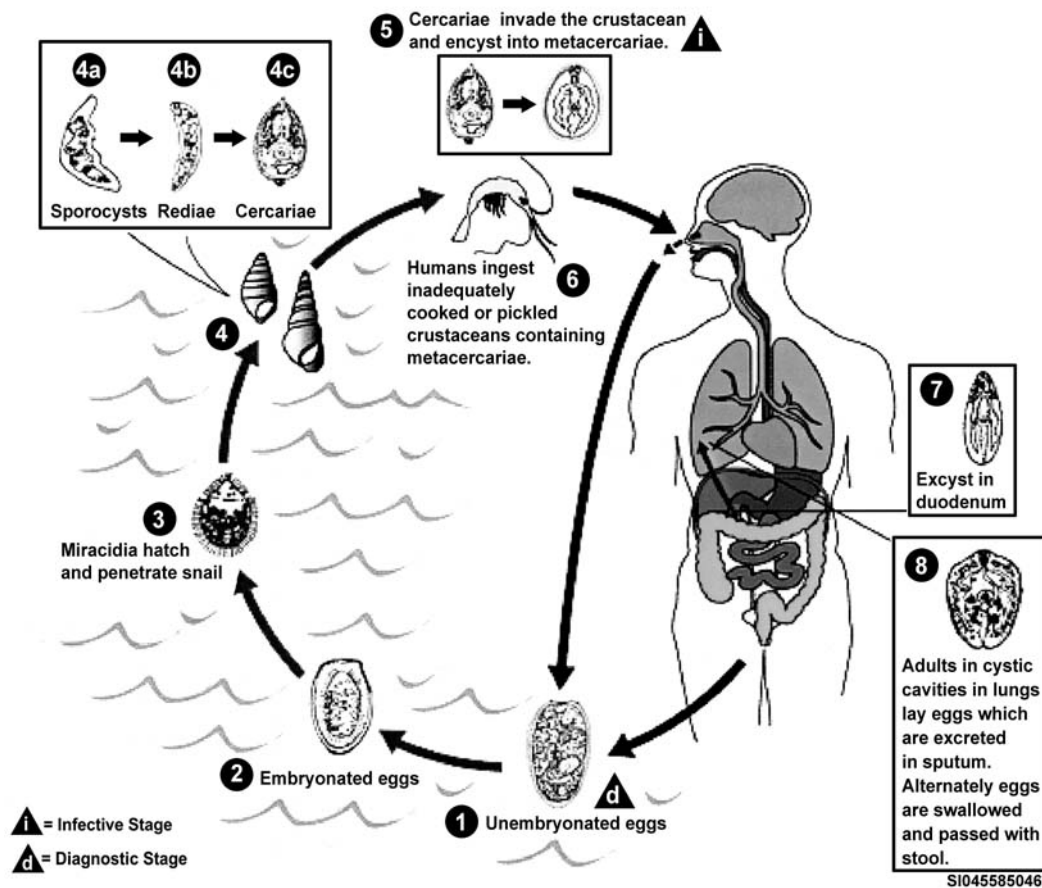


Fig. 2-24. Life cycle of *Paragonimus westermani*.

### Geographic Distribution

While *P. westermani* occurs in the Far East, other species of *Paragonimus* are encountered in Asia, the Americas, and Africa.

### Epidemiology

There are several species of *Paragonimus* that can parasitize the lungs of humans and other carnivores are found in various parts of the world, including Asia, Africa, and Latin America.

Probably the most important species causing human disease to be described is *P. westermani*, which is found throughout Asia.

### **Pathology and clinical manifestations**

The early, invasive stages of paragonimiasis cause few or no symptoms. While migrating through different organs and tissues, they produce localized hemorrhage and leukocytic infiltrates. Once in the lung or an ectopic site, the worm stimulates connective tissue proliferation that eventually will enshroud it in a brownish or bluish capsule with eosinophils and neutrophils. Such capsules often ulcerate and heal slowly. Worms in the spinal cord are known to produce paralysis, which is sometimes total. When the organism infects the heart, it can be fatal. Pulmonary cases are usually characterized by early morning coughing, breathing difficulty, and flecks described as “iron filings” found in the sputum. Also, hemoptysis (bloody sputum) is a common occurrence.

### **Laboratory diagnosis**

Finding the typical eggs in either the stool or the sputum, as shown in figure 2–25, which will be bloody, makes diagnosis. The broadly ovoid, operculate, thick-shelled, unembryonated egg measures 80 to 120  $\mu\text{m}$  long by 45 to 70  $\mu\text{m}$  wide. The operculum is flattened and is usually set off from the rest of the shell by prominent shoulders. The abopercular end is sometimes thickened, but does not have a knob. Size allows ready distinction from the eggs of *D. latum* and *Fasciola* or *Fasciolopsis*. Adult worms are thick, robust, and measure 7 to 12 mm long by 4 to 6 mm wide.

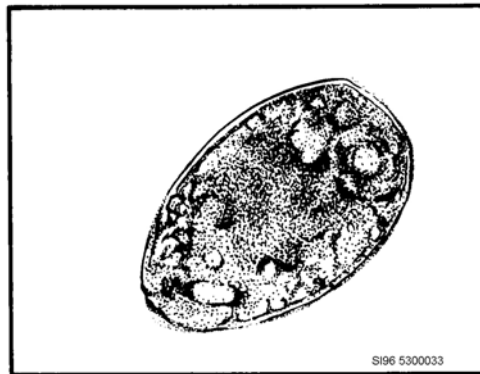


Fig. 2–25. Egg of *Paragonimus westermani*.

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## **Self-Test Questions**

After you complete these questions, you may check your answers at the end of the unit.

### **614. The schistosomes**

1. Where do adult schistosomes live in the human body?
2. What type of response is elicited from schistosome eggs by the human body?
3. What stage in the life cycle of schistosomes is liberated from the intermediate snail host?
4. What form, on contact with man, penetrates the skin?

5. How do you describe the schistosomes' route through the body once penetration is achieved by the cercariae?
6. Which is the most widely distributed of the five medically important schistosomes?
7. What symptoms are present during the prepatent period in a patient with *S. mansoni*?
8. What is one of the earliest and most common symptoms of a *S. haematobium* infection?
9. Once visualized, how are the eggs of the schistosomes differentiated?

**615. *Fasciolopsis buski* (giant intestinal roundworm)**

1. How does the life cycle of *F. buski* differ from many other trematodes?
2. What is the first intermediate host of *F. buski*?
3. What must the cercariae of *F. buski* do in order to mature into an infective metacercariae?
4. How are the symptoms of a patient with a heavy infection of *F. buski* characterized?
5. What is the most common method of diagnosing a *F. buski* infection?

**616. *Fasciola hepatica* (sheep liver fluke)**

1. Where does the adult *F. hepatica* live in the human body?
2. Where or in what countries is human infection with *F. hepatica* most common?
3. What are the pathological and clinical manifestations of human fascioliasis?
4. How can you differentiate the spurious presence of eggs in a patient's sample from a true infection?

**617. *Clonorchis sinensis* (oriental liver fluke) and *Opisthorchis* species**

1. How are human infections of *Clonorchis* and *Opisthorchis* acquired?
2. How do the eggs of *Clonorchis* and *Opisthorchis* leave the human body?
3. How long does the complete life cycle of *Clonorchis* and *Opisthorchis* from person-to-snail to fish-to-person take?
4. Clonorchiasis and opisthorchiasis are considered what type of parasitic infections?
5. What are some factors that favor human infection with *Clonorchis* and *Opisthorchis*?
6. Why is the use of concentration procedures strongly recommended for the recovery of *Clonorchis* and *Opisthorchis*?
7. What generic diagnosis is reported based on the recovery of eggs of the *Clonorchis/Opisthorchis* type that cannot be differentiated?

**618. *Paragonimus westermani* (oriental lung fluke)**

1. Where are adult *P. westermani* usually found in the human body?
2. What are some ectopic sites where *P. westermani* may also be found?
3. *P. westermani* eggs may exit the body in what two specimens?
4. What is the second intermediate host of *P. westermani*?
5. How does man become infected with *P. westermani*?



6. What are the symptoms of pulmonary cases of *P. westermani*?

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## Answers to Self-Test Questions

### 606

1. Infection occurs when eggs containing third-stage larvae are ingested.
2. 10 days.
3. Cool to temperate regions.
4. Children.
5. Localized itching and behavioral changes.
6. Vulvovaginitis, salpingitis, and pelvic granuloma.
7. Finding eggs on cellulose tape preparations; six.
8. The eggs are elongated with a thick colorless shell, flattened on one side, range in size from 50 to 60  $\mu\text{m}$  long by 20 to 40  $\mu\text{m}$  wide, and contain infective larvae.

### 607

1. Ingestion of fully embryonated eggs.
2. 90 days.
3. *Ascaris* and/or hookworm.
4. Light infections are usually asymptomatic, moderate infections cause bloody diarrhea, and heavy infections result in malnutrition and dysentery with bloody mucoid stools.
5. Eggs, many eosinophils, and Charcot-Leyden crystals.
6. Demonstration of eggs in feces or by sigmoidoscopic observation of worms.
7. Eggs of *Trichuris* passed in stool specimens are yellowish-brown, thick shelled, and barrel-shaped, with clear, mucoid polar plugs at each end. They are 50 to 55  $\mu\text{m}$  long to 22 to 24  $\mu\text{m}$  wide.
8. *C. philippinensis* eggs are smaller, broader, more ovoid and have a striated shell.

### 608

1. Months or even years.
2. 8 to 12 weeks.
3. No.
4. Bowel or bile duct, pancreatic duct, or appendix obstruction.
5. Fertilized mammillated, fertilized un-mammillated, unfertilized mammillated, and unfertilized un-mammillated.
6. Adults are white or pinkish; females are 20 to 35 cm in length and 3 to 6 mm in diameter; and males are roughly 115 to 31 cm long and 2 to 4 mm in diameter.
7. Because of the large number of eggs passed; on average 200,000 per day for each female.

### 609

1. The third stage filariform larvae.
2. By penetrating the skin or by ingestion.
3. 5 to 10 years.
4. Infection of human population, defecation onto soil, acceptable environmental conditions (temperature, rainfall pattern, and open, sandy soil), and human contact with the infective larvae in soil.
5. Hypochromic microcytic.
6. Iron deficiency anemia.
7. *A. duodenale*.

8. No.
9. Set up a “Harada-Mori” culture and examine the buccal structures of the larvae.
10. Perform a simple egg count by making a direct saline wet mount from a fresh specimen and systematically examine the entire smear and count all the eggs.
11. In herbivores; humans are infected by ingesting the contaminated plant material.

**610**

1. Indirect.
2. Drier and cooler conditions.
3. The infective larvae penetrate the skin.
4. The tunneling process in the small intestines and tissue reaction to eggs, worms, and larvae.
5. Feces and duodenal contents.
6. The buccal canal of *Strongyloides* is very short, while that of the hookworm is longer and narrow and the genital primordium of *Strongyloides* is much larger than that in the hookworm.

**611**

1. By direct fecal-oral ingestion of eggs.
2. 2 to 3 weeks.
3. *H. nana*.
4. Rat or mice fleas as well as grain beetles.
5. Its cysticercoid cannot develop at the higher temperatures encountered in a mammalian intermediate host.
6. Members of the genus *Tribolium* (grain and flour beetles).
7. *H. nana* (dwarf tapeworm).
8. Moderate to profuse diarrhea, nervous disorders, abdominal pain, and, in some cases, extreme apathy.
9. The difference in size.

**612**

1. Cattle for *T. saginata* and pigs for *T. solium*.
2. *T. solium*.
3. *T. saginata* has four suckers, while *T. solium* has four suckers plus two rows of hooks located at the anterior end of its scolex.
4. On passage from the body.
5. The passage of motile proglottids.
6. It can be fatal.
7. Eggs or gravid proglottids passed in the feces or collected from the perianal fold.
8. *Taenia* species found.
9. Gravid proglottids recovered from a fresh specimen.

**613**

1. A crustacean and fish.
2. By eating raw, poorly cooked, or pickled freshwater fish.
3. The worm can be up to 10 to 15 meters long, with as many as 3,000 proglottids.
4. Vitamin B<sub>12</sub> deficiency anemia.
5. Diarrhea, obstruction of the bile duct or intestine, and toxic symptoms.
6. Ivory in color, wider than long, having a rosette-shaped uterus with a central uterine pore.
7. The eggs are moderately thick-shelled, yellowish-brown in color, oval, measuring 58 to 75  $\mu\text{m}$  by 44 to 50  $\mu\text{m}$ , and filled with yolk cells.

**614**

1. In the smaller venules of the mesenteric and vesicle plexuses.

2. A marked inflammatory reaction.
3. Cercariae.
4. Cercariae.
5. They enter the circulation and pass through the lungs to establish themselves in the liver for a short period of maturation, and then they migrate via the portal system to the mesenteric or vesicle venules.
6. *S. mansoni*.
7. Urticarial skin rash, fever, eosinophilia, or diarrhea, and the liver may be enlarged and tender.
8. Hematuria.
9. Size and position of their characteristic spines.

**615**

1. Its second intermediate host is any of several aquatic plants.
2. A planorbis snail.
3. Encyst on aquatic vegetation.
4. Traumatic, obstructive, or toxic.
5. Microscopic identification of the eggs in the patient's feces.

**616**

1. The bile ducts.
2. Parts of Europe, northern Africa, Cuba, South America, and other sheep or cattle-raising locales worldwide.
3. Headache, chills, fever, and right upper quadrant pain with hepatomegaly.
4. By having the patient abstain from eating liver for 3 days and then performing another stool examination.

**617**

1. By ingesting raw or improperly cooked fish containing the cyst-like larval stage of the parasite.
2. Carried with the bile into the intestines and leave the body in the feces.
3. At least 3 months.
4. Zoonotic.
5. Eating habits and the use of night soil in the commercial production of fish.
6. Due to the relatively low egg production.
7. *Clonorchis/Opisthorchis/Heterophyes/Metagonimus* species may be reported.

**618**

1. The lungs.
2. Lymph nodes, brain, and peritoneal cavity.
3. Sputum and feces.
4. Crab or crayfish.
5. By eating raw or improperly cooked crabs or crayfish.
6. Early morning coughing, difficulty breathing, and iron filings found in the sputum.

**Do the unit review exercises before going to the next unit.**

## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter.

14. (606) The **most** common nematode infecting humans in the United States is the
  - a. *Trichuris trichiura*.
  - b. *Trichinella spiralis*.
  - c. *Ancylostoma duodenale*.
  - d. *Enterobius vermicularis*.
15. (606) The two symptoms associated with *Enterobius vermicularis* infections are
  - a. nausea and abdominal pain.
  - b. nausea and behavioral changes.
  - c. localized itching and abdominal pain.
  - d. localized itching and behavioral changes.
16. (607) How many weeks does it take for *T. trichiura* eggs to embryonate and become infective after passage from the body?
  - a. One.
  - b. Two.
  - c. Three.
  - d. Four.
17. (607) *T. trichiura* dysentery may have to be differentiated from that caused by
  - a. *Necator americanus*.
  - b. *Ascaris lumbricoides*.
  - c. *Dientamoeba fragilis*.
  - d. *Entamoeba histolytica*.
18. (607) The eggs of *T. trichiura* *must* be differentiated from those of
  - a. *D. latum*.
  - b. *C. sinensis*.
  - c. *Taenia* species.
  - d. *C. philippinensis*.
19. (608) How many weeks after ingestion of *A. lumbricoides* are sexually mature adults found in the intestines?
  - a. 2 to 4.
  - b. 4 to 6.
  - c. 8 to 12.
  - d. 12 to 16.
20. (608) What type of *Ascaris lumbricoides* eggs are too dense to be recovered by flotation procedures?
  - a. Fertile.
  - b. Infertile.
  - c. Mammillated.
  - d. Unmammillated.
21. (609) What stage of the filariform hookworm larvae is infective?
  - a. First.
  - b. Second.
  - c. Third.
  - d. Fourth.

- 
- 
22. (609) What hookworm produces a more severe disease?
- Necator americanus*.
  - Ancylostoma caninum*.
  - Ancylostoma duodenale*.
  - Ancylostoma ceylanicum*.
23. (609) What type of culture can be set up to aid in identification of a hookworm species?
- Slide.
  - Charcoal.
  - Harada-Mori.
  - Novy MacNeal Nicolle.
24. (610) When rhabditiform larvae of the *Strongyloides stercoralis* follow the indirect route of development, they
- are deposited on dry, cool soil.
  - live as long as 2 months in the soil.
  - develop into free-living adult male and female worms.
  - develop into infective filariform larvae within 24 hours after leaving the intestine.
25. (610) The point differentiating the rhabditiform larvae of *Strongyloides* from those of hookworms is the
- notched tail of hookworm larva.
  - pointed tail of *Strongyloides* larva.
  - very short buccal canal of *Strongyloides*.
  - much smaller genital primordium of *Strongyloides*.
26. (611) What life cycle of *Hymenolepis nana* requires an intermediate host, and which of the following hosts are acceptable?
- External; fly.
  - Modified; snail.
  - Indirect; rat flea.
  - Direct; grain beetle.
27. (611) Spherical to ovoid, hexacanth oncosphere, and polar filaments are terms used to describe the eggs of
- Taenia solium*.
  - Taenia saginata*.
  - Hymenolepis nana*.
  - Diphyllobothrium latum*.
28. (611) In comparison to *Hymenolepis nana*, the ova of *Hymenolepis diminuta* are
- larger.
  - smaller.
  - the same size.
  - extremely variable in size.
29. (612) The intermediate hosts for *Taenia saginata* are
- rabbit.
  - pigs.
  - sheep.
  - cattle.

30. (612) What tapeworm larvae is the causative agent of cysticercosis, a serious disease?
- a. *Taenia solium*.
  - b. *Taenia saginata*.
  - c. *Hymenolepis nana*.
  - d. *Hymenolepis diminuta*.
31. (612) How many lateral uterine branches does the gravid proglottid of the *Taenia solium* have?
- a. 3 to 5.
  - b. 5 to 7.
  - c. 7 to 13.
  - d. 13 to 17.
32. (613) *D. latum* can be as long as
- a. 1 to 5 feet.
  - b. 10 to 15 feet.
  - c. 1 to 5 meters.
  - d. 10 to 15 meters.
33. (613) The proglottids of *D. latum* are ivory in color, wider than long, and have
- a. a starburst uterus.
  - b. a daisy-shaped uterus.
  - c. an umbrella-like uterus.
  - d. a rosette-shaped uterus.
34. (614) What medically important schistosomes is **most** widely distributed?
- a. *S. mekongi*.
  - b. *S. mansoni*.
  - c. *S. japonicum*.
  - d. *S. haematobium*.
35. (614) Parasite eggs that are smaller than the other species and have a minute lateral spine that may or may not be visible, best describes
- a. *S. mansoni*.
  - b. *S. mekongi*.
  - c. *S. japonicum*.
  - d. *S. haematobium*.
36. (615) Diagnosis of *F. buski* is *most* commonly based on
- a. recovery of typical eggs in the feces.
  - b. recovery of typical eggs in the sputum.
  - c. symptoms much like those of a duodenal ulcer.
  - d. intestinal obstruction and a profound toxic reaction.
37. (616) Adult *F. hepatica* are normally found in the human body in the
- a. bile ducts.
  - b. large intestine.
  - c. alveoli of the lungs.
  - d. fine venules surrounding the small intestines.
38. (616) When the presence of spurious *Fasciola hepatica* is suspected, the patient is instructed to
- a. submit a purged specimen.
  - b. submit six consecutive stool specimens.
  - c. immediately submit an additional stool specimen.
  - d. abstain from eating liver for three days prior to a repeat stool examination.

39. (617) *C. sinensis* and *O. viverrini* require **at least** how long to complete their life cycles?
- a. 2 weeks.
  - b. 1 month.
  - c. 3 months.
  - d. 6 months.
40. (617) The **usual** primary hosts of *C. sinensis* and *O. viverrini* are
- a. fish and snakes.
  - b. snails and worms.
  - c. humans and birds.
  - d. domestic and wild fish-eating animals.
41. (617) The basic pathologic change seen in patients with clonorchiasis or opisthorchiasis is
- a. obstruction of the bowel.
  - b. scarring of the bladder wall.
  - c. thickening of the mesenteric blood vessels.
  - d. inflammation of the lining of the bile ducts.
42. (618) Adult *Paragonimus westermani* **usually** live in the human body in the
- a. lungs, encapsulated in pairs.
  - b. liver, surrounded by a hydatid cyst.
  - c. smallest branches of the biliary tree.
  - d. large intestines, encapsulated in a sac of serous fluid.
43. (618) Adult *Paragonimus westermani* organisms have been found in all the following sites, **except** the
- a. brain.
  - b. intestines.
  - c. lymph nodes.
  - d. peritoneal cavity.
44. (618) A common symptom of *Paragonimus westermani* infections is
- a. paralysis.
  - b. bloody urine.
  - c. obstruction of the biliary tree.
  - d. hemoptysis (bloody sputum).

## **Student Notes**



## Unit 3. Tissue and Blood Helminths

|                                        |     |
|----------------------------------------|-----|
| 619. Tissue Helminths .....            | 3–1 |
| 620. Blood Helminths—The Filariae..... | 3–8 |

**A**LL the major classes of the helminths have species that can be considered tissue and/or blood parasites. They can live in tissues or blood as adult worms or larvae. Most of these organisms are zoonotic species; however, some are natural human parasites. Diagnosis is made by the identification of reproductive products found in blood, feces, other body fluids, or, in case of the larval parasites, the recovery of the parasite itself from tissues. Many of these organisms are rare in the United States, but from a worldwide perspective these exotic organisms are of great concern to public health officials and the World Health Organization (WHO).

### 619. Tissue Helminths

The tissue helminths represent a diverse group of nematodes, cestodes, and trematodes. Some of these organisms are quite rare. Others were discussed in unit 2 because their eggs are passed in feces and urine. In this section, we'll restrict our study to the most common parasites that *cannot* be diagnosed by microscopic examination of feces or urine specimens.

#### *Trichinella spiralis* and other tissue helminths

The nematode *T. spiralis* is a parasite of carnivores and is the common agent of human trichinosis. It was first seen in human tissue at autopsy in the early 1800s, but, it was not until 1860 that Friedrich von Zenker concluded that the infection resulted from eating raw sausage. In the 1900s, it was recognized as a definite public health problem.

#### *Trichinella spiralis* nematodes

*Trichinella* is unique among the nematodes that parasitize humans in that its life cycle does not include any developmental stage outside the body of a host, and it does not involve a true intermediate host. This life cycle is illustrated in figure 3–1.

#### Causal Agents

Trichinellosis (trichinosis) is caused by nematodes (roundworms) of the genus *Trichinella*. In addition to the classical agent *T. spiralis* (found worldwide in many carnivorous and omnivorous animals), several other species of *Trichinella* are now recognized, including *T. pseudospiralis* (mammals and birds worldwide), *T. nativa* (Arctic bears), *T. nelsoni* (African predators and scavengers), and *T. britovi* (carnivores of Europe and western Asia).

Trichinellosis is acquired by ingesting meat containing cysts (encysted larvae) of *Trichinella*. After exposure to gastric acid and pepsin, the larvae are released ② from the cysts and invade the small bowel mucosa where they develop into adult worms (female 2.2 mm in length, males 1.2 mm; life span in the small bowel: 4 weeks). After 1 week, the females release larvae that migrate to the striated muscles where they encyst. *Trichinella pseudospiralis*, however, does not encyst. Encystment is completed in 4 to 5 weeks and the encysted larvae may remain viable for several years. Ingestion of the encysted larvae perpetuates the cycle. Rats and rodents are primarily responsible for maintaining the endemicity of this infection. Carnivorous/omnivorous animals, such as pigs or bears, feed on infected rodents or meat from other animals. Different animal hosts are implicated in the life cycle of the different species of *Trichinella*. Humans are accidentally infected when eating improperly processed meat of these carnivorous animals (or eating food contaminated with such meat).

#### Geographic Distribution

Worldwide. Most common in parts of Europe and the United States.

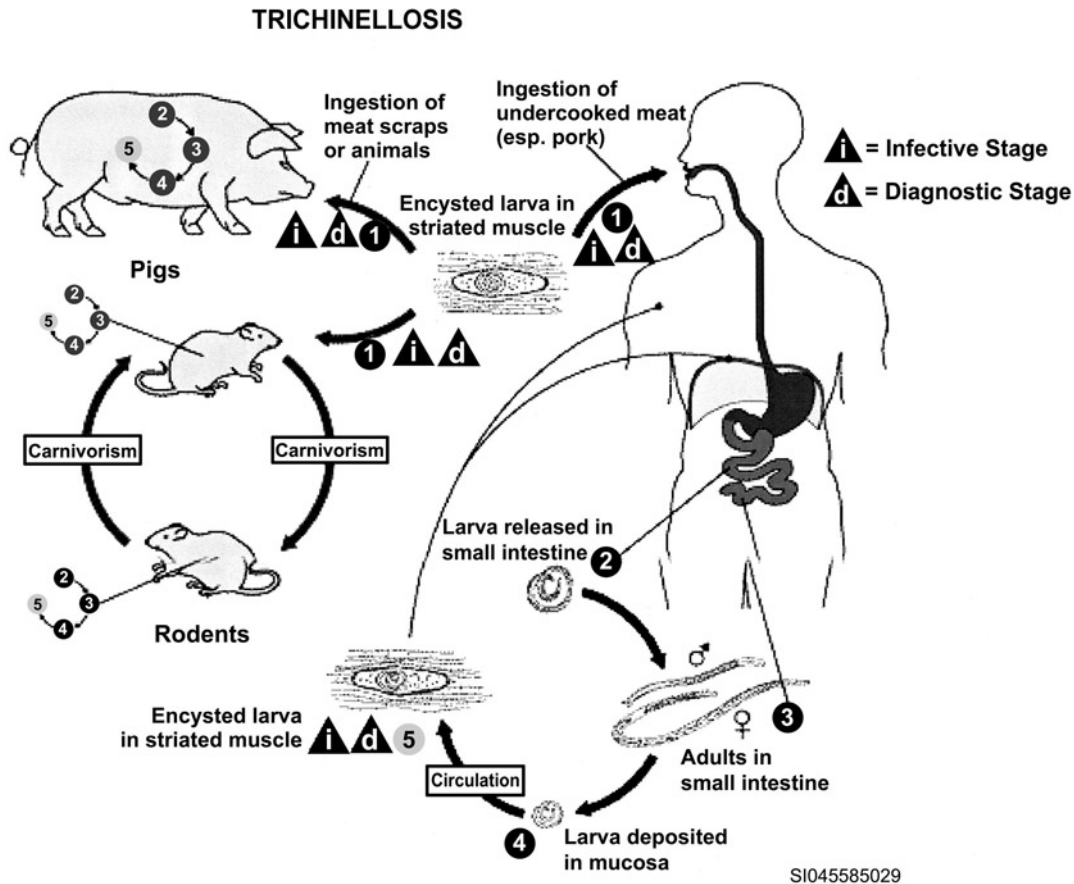


Figure 3-1. Life cycle of *Trichinella spiralis*.

### Life cycle

*T. spiralis* develops about equally well in humans as it does in pigs, rats, and many other mammals. Usually, humans acquire *Trichinella* infections by eating raw or poorly cooked pork, bear, walrus, horse meat, or meat from other carnivores that contain viable infective larvae. The human cycle begins when meat is digested in the stomach where the cyst surrounding the larvae is dissolved. In the small intestine, the excysted larvae enter the intestinal mucosa. The larvae mature and mate by the second day. The adults are small, white worms, just visible to the unaided eye. The male is 1.4 to 1.6 mm long by 40 to 60  $\mu$ m wide, while the female measures 2.5 to 4.0 mm long by 100 to 150  $\mu$ m wide. The male dies after mating and is passed from the intestine in a very short time. In contrast, upon fertilization, the female burrows more deeply into the intestinal mucosa, and by the 5th to 7th day, she begins to deposit motile larvae directly into the mucosa. A single female gives birth to several hundred larvae over a period of 4 to 16 weeks. The minute larvae measure 100  $\mu$ m long and 6  $\mu$ m in diameter. They reach the intestinal lymphatic system or mesenteric venules and are distributed to all parts of the body. When the young larvae leave the capillaries, they invade the voluntary (striated) muscle. This is the only tissue in which the larvae are able to develop and grow. The most frequently affected muscles are in the diaphragm, larynx, tongue, jaws, neck, ribs, biceps, gastrocnemius, and deltoids. After 1 to 2 weeks of exposure, most of the larvae have reached the striated muscle. About 3 weeks after exposure, the larvae in the muscles have grown to about 1 mm in length. At this stage, they have become coiled, and encapsulation has begun. The cyst wall results from the host's immune response to the presence of the larvae. The encysted larvae are now infectious. Calcification of the capsule begins after about 8 weeks. In 9 to 12 months, most of the encapsulated larvae are completely calcified and dead, but some may live for several years.

## Epidemiology

*Trichinella* has a worldwide distribution. The principal reservoir for human infection is the pig. As expected, the parasite is found most commonly in pork-eating populations. Until the 1950s, *Trichinella* was traditionally considered a serious problem in parts of Europe and the United States. It is still widely seen in Germany, Poland, Spain, Hungary, and the lower Danube countries. Also, there are reports of small epidemic outbreaks still occurring in the United States and Latin America.

## Pathology and clinical manifestations

Pathological changes are classified as intestinal effects and muscle penetration and larvae encapsulation. The severity of the disease caused by *Trichinella spiralis* is related to the number of ingested cyst. Ingestion of a small dose of cyst probably goes unrecognized by the body. In heavier infections, as the young worms excyst and migrate into the intestinal mucosa, the patient exhibits symptoms of gastroenteritis or food poisoning. As the worms mature and release young larvae, acute inflammatory reactions occur around the larvae as they become temporarily trapped in capillaries of various organs during their migration through the tissues. An eosinophilia of 20 to 50 percent develops during this period. Patients with severe infections may experience symptoms affecting special muscle groups, such as painful swallowing, breathing, or chewing. In very heavy infections, as larvae continue to be produced and invade muscles, the outcome can be fatal or permanent crippling. It is estimated that 10 to 20 percent of the patients have central nervous system involvement, and, if not treated, the mortality rate for these patients may reach 50 percent.

## Laboratory diagnosis

A definitive diagnosis of *Trichinella* is very difficult to make in the early stages of the disease. Often the first clue for the physician is the patient's history of possible ingestion of raw or rare pork or other meat. The patient may also exhibit peripheral eosinophilia. Since it is very rare to recover adult worms or larvae from feces or other body fluids, finding encapsulated larvae in the muscle makes definitive diagnosis. A muscle biopsy is performed and a portion of the tissue is pressed between two glass slides. Microscopic examination of the tissue reveals the easily seen encapsulate larvae. If a large amount of tissue is available, it can be digested with gastric juices and the sediment examined for freed *Trichinella spiralis* larvae. Also, larvae can usually be found in the suspected meat if it is still available. Use the compression slide technique for examining suspected meat as well as for examining muscle biopsies. Skin test antigens are available for *Trichinella*, and the physician will occasionally use these as an aid in diagnosis. *T. spiralis* is a highly antigenic parasite, therefore, reliable serological procedures are also available. The bentonite flocculation (BF) test is the standard method. Serological procedures, when performed on acute and convalescent serum, will show a significant increase in titer.

## Miscellaneous nematode tissue parasites

The list of miscellaneous nematode tissue parasites include, but, not limited to, *Toxocara canis*, *Toxocara cati*, *Ancylostoma braziliense*, *Ancylostoma caninum*, *Dracunculus medinensis*, *Angiostrongylus cantonensis*, *Angiostrongylus costaricensis*, *Gnathostoma spinigerum*, and *Capillaria hepatica*. These infections are acquired by ingestion of; fecal material from soil, infected copepod, water fleas, slugs, snails, frogs, snakes, and poorly cooked fish or chicken. Also, a few are acquired through skin penetration of infective larvae. They invade the skin, feet, ankles, liver, lungs, eyes, brain, and walls of the intestines. Diagnosis is made by serological methods, radiological studies (X-rays, CT scans, etc.) of calcified worms, and examination of cutaneous lesions, biopsies, and autopsy tissue specimens.

## Causal Agents

Toxocariasis is caused by larvae of *Toxocara canis* (dog roundworm) and less frequently of *T. cati* (cat roundworm), two nematode parasites of animals.

*Toxocara canis* accomplishes its life cycle in dogs (fig. 3-2), with humans acquiring the infection as accidental hosts. Following ingestion by dogs, the infective eggs hatch and larvae penetrate the gut wall and migrate into various tissues, where they encyst if the dog is older than 5 weeks. In younger dogs, the larvae migrate through the lungs, bronchial tree, and esophagus; adult worms develop and oviposit in the small intestine. In the older dogs, the encysted stages are reactivated during pregnancy, and infect by the transplacental and transmammary routes the puppies, in whose small intestine adult worms become established. Thus, infective eggs are excreted both by lactating bitches and puppies. Humans are accidental hosts who become infected by ingesting infective eggs in contaminated soil. After ingestion, the eggs hatch and larvae penetrate the intestinal wall and are carried by the circulation to a wide variety of tissues (liver, heart, lungs, brain, muscle, eyes). While the larvae do not undergo any further development in these sites, they can cause severe local reactions that are the basis of toxocariasis. The two main clinical presentations of toxocariasis are visceral larva migrans (VLM) and ocular larva migrans (OLM). *Baylisascaris procyonis*, a roundworm of raccoons, has been reported to cause similar VLM and OLM syndromes in humans.

### Geographic Distribution

*Toxocara canis* is found worldwide.

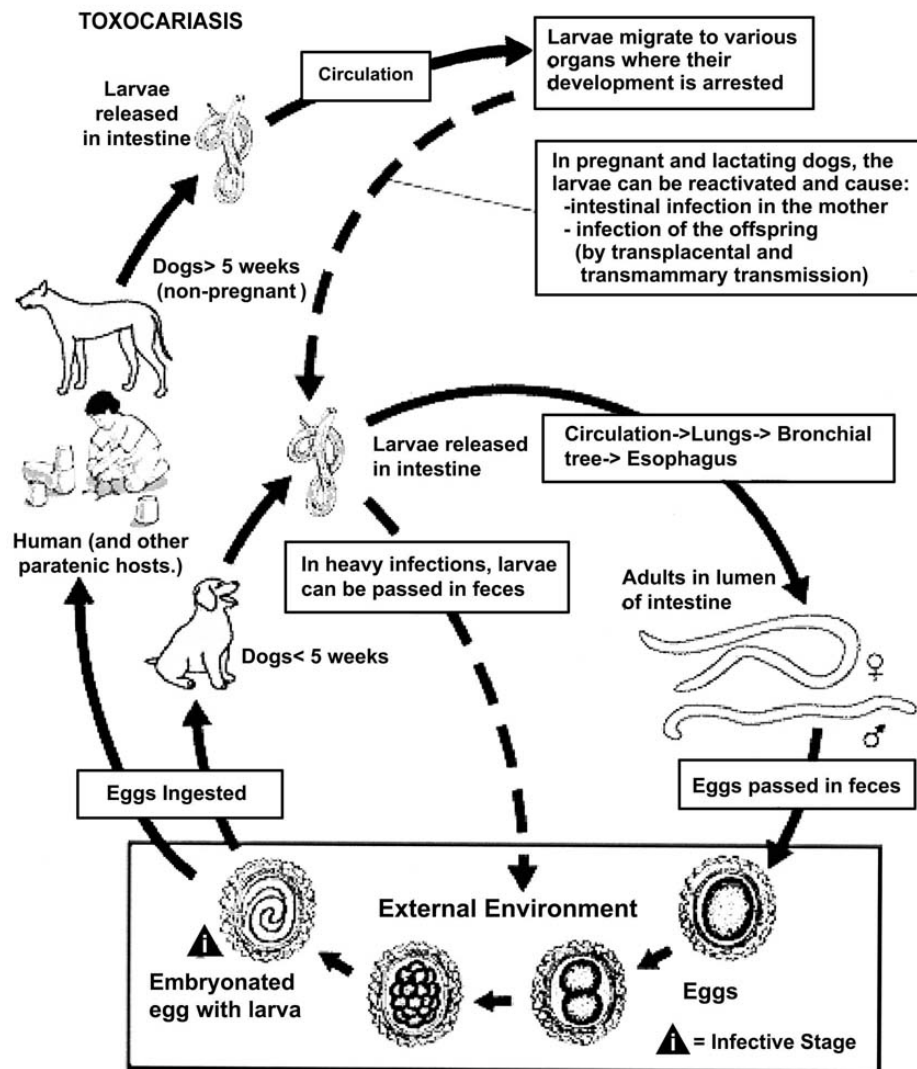


Figure 3-2. Life cycle of *Toxocara*.

### Causal Agent

The nematode (roundworm) *Gnathostoma spinigerum* and *Gnathostoma hispidum*, infects vertebrate animals. Human gnathostomiasis is due to migrating immature worms.

Refer to figure 3-3 for the following references. In the natural definitive host (pigs, cats, dogs, wild animals) the adult worms reside in a tumor which they induce in the gastric wall. They deposit eggs that are unembryonated when passed in the feces. ① Eggs become embryonated in water, and eggs release first-stage larvae ②. If ingested by a small crustacean (*Cyclops*, first intermediate host), the first-stage larvae develop into second-stage larvae ③. Following ingestion of the *Cyclops* by a fish, frog, or snake (second intermediate host), the second-stage larvae migrate into the flesh and develop into third-stage larvae ④. When the second intermediate host is ingested by a definitive host, the third-stage larvae develop into adult parasites in the stomach wall. Alternatively, the second intermediate host may be ingested by the paratenic host (animals such as birds, snakes, and frogs) in which the third-stage larvae do not develop further but remain infective to the next predator ⑥. Humans become infected by eating undercooked fish or poultry containing third-stage larvae, or reportedly by drinking water containing infective second-stage larvae in *Cyclops* ⑦.

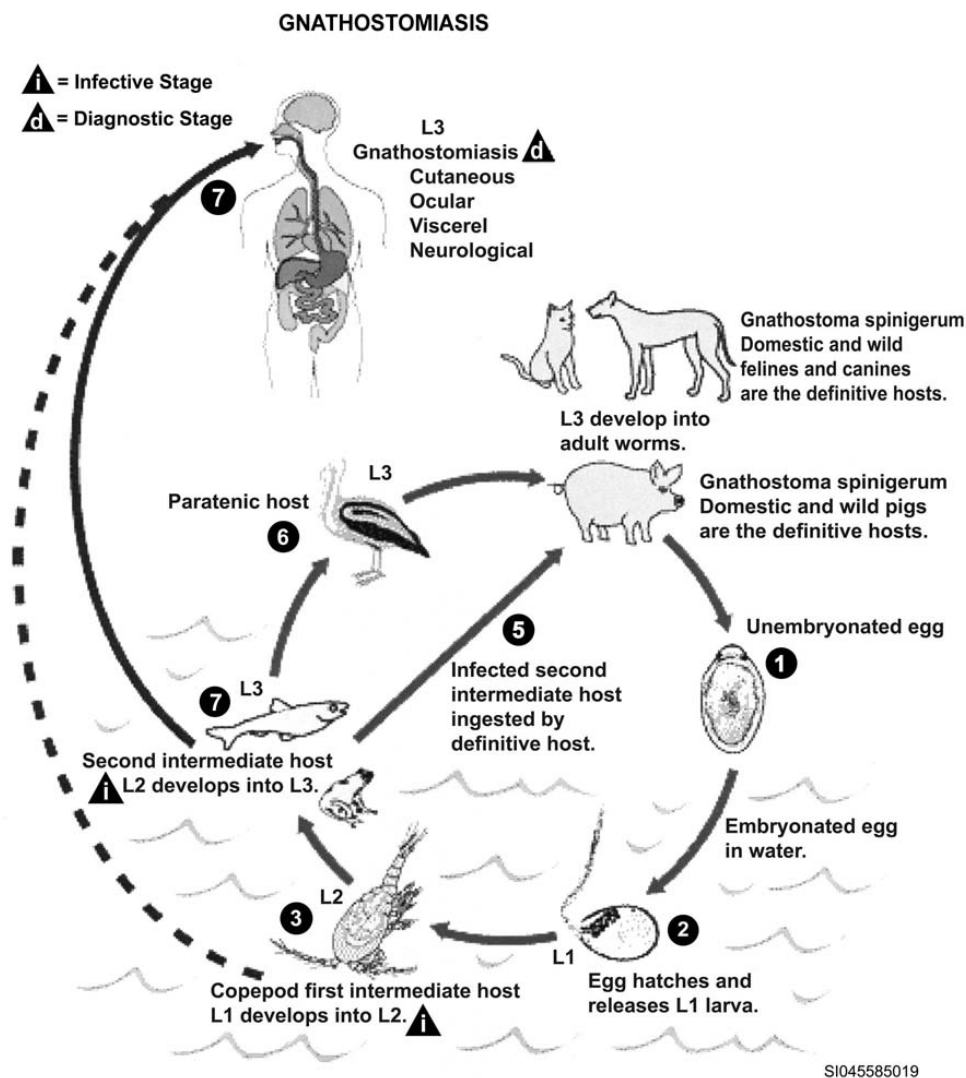


Figure 3-3. Life cycle of *Gnathostoma*.

**Geographic Distribution**

Asia, especially Thailand and Japan; recently emerged as an important human parasite in Mexico.

**Echinococcus granulosus cestode**

*E. granulosus* is a cestode that is the causative agent of hydatidosis or hydatid disease. Hydatid cysts were recognized by Hippocrates, Galen, and Areatus – according to historical information. In the 1600s and 1700s, there was speculation concerning the possible relationship between humans and animal hydatid cysts.

**Life cycle**

Sheep and cattle are considered intermediate hosts and humans are accidental intermediate host. *E. granulosus* is minute tapeworm that lives in the small intestines of domestic and wild canines. Infection occurs by ingesting the typical taeniid eggs excreted in the feces of infected dogs (definitive hosts). Once ingested, oncospheres are liberated from the eggs and migrate via the bloodstream to the liver, lungs, and other tissues and organs to develop into hydatid cysts. The hydatid cysts contain brood capsules and protoscoleces, as was seen back in unit 1 (fig.1–7), which begin to grow and develop slowly within the cyst. Ultimately the cyst may become very large and contain hundreds or thousands of protoscoleces, which are potentially infective organisms.

**Epidemiology**

*E. granulosus* is distributed worldwide in sheep- and cattle-raising areas. It is more common in Australia, New Zealand, southern Africa, southern South American, parts of Europe, with sporadic foci in North America and the Orient.

**Pathology and clinical manifestations**

Hydatid disease in humans is potentially dangerous. Because the cysts grow slowly, they may go undetected for many years until they reach sufficient size to cause clinical manifestations. The majority of hydatid cysts appear in the liver and produce chronic abdominal pain or a visible abdominal mass. If the cysts are in vital organs, central nervous system, or bones, even small cysts can cause severe damage in these areas. Cysts in the lungs are usually asymptomatic but can produce a cough, shortness of breath, or chest pain. During the life cycle of the cyst, there may be small fluid leaks into the circulatory system. The patient becomes sensitized from the small leaks and if the cyst should burst, a serious allergic reaction may occur.

**Laboratory diagnosis**

Many asymptomatic cysts are first discovered after X-rays are taken. Serological procedures are also available for detection of *Echinococcus*. Once the cyst is discovered and surgical removal is selected as the approach of removal, some of the cyst fluid can be aspirated. However, this procedure is not without risk due to possible fluid and/or tissue leakage or dissemination. Cyst aspiration should only be performed at the time of surgery. The aspirate is submitted for microscopic examination in order to detect the presence of hydatid sand, as you saw back in unit 1 (fig. 1–7), thus confirming the diagnosis.

**Causal Agent**

Human echinococcosis (hydatidosis, or hydatid disease) is caused by the larval stages of cestodes (tapeworms) of the genus *Echinococcus*. *Echinococcus granulosus* causes cystic echinococcosis, the form most frequently encountered; *E. multilocularis* causes alveolar echinococcosis; *E. vogeli* causes polycystic echinococcosis; and *E. oligarthrus* is an extremely rare cause of human echinococcosis.

Refer to figure 3–4 for the following references. The adult *Echinococcus granulosus* (3 to 6 mm long) ❶ resides in the small bowel of the definitive hosts, dogs or other canids. Gravid proglottids release eggs ❷ that are passed in the feces. After ingestion by a suitable intermediate host (under natural conditions: sheep, goat, swine, cattle, horses, camel), the egg hatches in the small bowel and releases



an oncosphere <sup>3</sup> that penetrates the intestinal wall and migrates through the circulatory system into various organs, especially the liver and lungs. In these organs, the oncosphere develops into a cyst <sup>4</sup> that enlarges gradually, producing protoscolices and daughter cysts that fill the cyst interior. The definitive host becomes infected by ingesting the cyst-containing organs of the infected intermediate host. After ingestion, the protoscolices <sup>5</sup> evaginate, attach to the intestinal mucosa <sup>6</sup>, and develop into adult stages <sup>1</sup> in 32 to 80 days. The same life cycle occurs with *E. multilocularis* (1.2 to 3.7 mm), with the following differences: the definitive hosts are foxes, and to a lesser extent dogs, cats, coyotes and wolves; the intermediate host are small rodents; and larval growth (in the liver) remains indefinitely in the proliferative stage, resulting in invasion of the surrounding tissues. With *E. vogeli* (up to 5.6 mm long), the definitive hosts are bush dogs and dogs; the intermediate hosts are rodents; and the larval stage (in the liver, lungs and other organs) develops both externally and internally, resulting in multiple vesicles. *E. oligarthrus* (up to 2.9 mm long) has a life cycle that involves wild felids as definitive hosts and rodents as intermediate hosts. Humans become infected by ingesting eggs <sup>2</sup>, with resulting release of oncospheres <sup>3</sup> in the intestine and the development of cysts <sup>4</sup>, <sup>4</sup>, <sup>4</sup>, <sup>4</sup> in various organs.

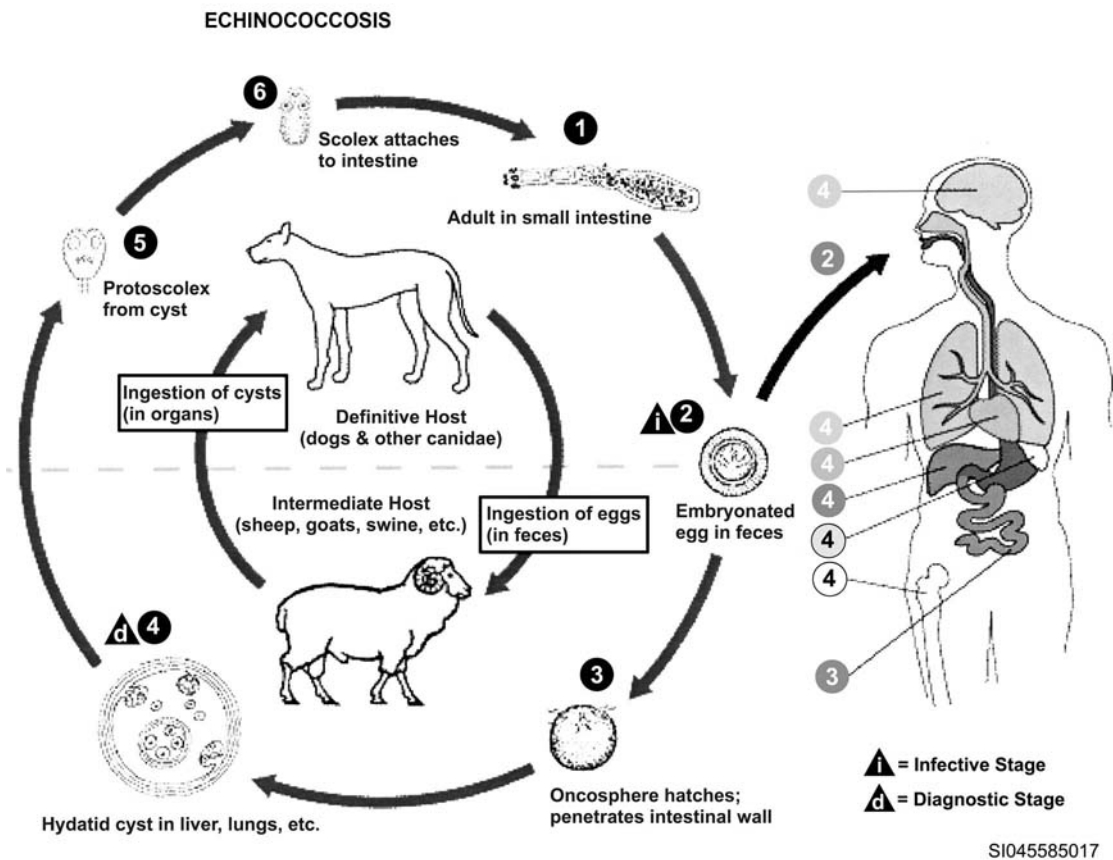


Figure 3-4. Life cycle of *Echinococcus*.

### Geographic Distribution

*E. granulosus* occurs practically worldwide, and more frequently in rural, grazing areas where dogs ingest organs from infected animals. *E. multilocularis* occurs in the northern hemisphere, including central Europe and the northern parts of Europe, Asia, and North America. *E. vogeli* and *E. oligarthrus* occur in Central and South America.

## 620. Blood Helminths—The Filariae

Filarial worms are arthropod-transmitted parasites that live the blood and lymphatic circulatory systems, muscles, connective tissues, and body cavities of vertebrates. All filariae have a unique characteristic; the adult female worm produces a primitive or pre-larva called a *microfilaria*. The microfilariae are found in the peripheral blood or skin. These organisms, although not found in the United States, are responsible for a significant amount of disease worldwide and have the potential to be imported into the United States. There are over 200 species of filarial parasites, but only few are known to infect humans. Of the human species, three species (*Wuchereria bancrofti*, *Brugia malayi*, and *Onchocerca volvulus*) account for most infections. Two-thirds of the 90 million people currently infected, live in China, India, and Indonesia. As early as 600 B.C. ancient Hindu and Persian physicians noted elephantiasis, which was probably due to *W. bancrofti*. *W. bancrofti* is the causative agent of bancroftian filariasis or elephantiasis and is the most common species of filaria infecting humans.

### Life cycle

True filarial worms, such as *W. bancrofti*, are unique because they have a microfilaria stage as shown in figure 3–6. The females deposit the microfilaria directly into the tissues. The adult worms live chiefly in the lymphatic system and occasionally in the blood. The microfilariae circulate in the blood. In some species, the microfilariae retain the egg membrane as a sheath (sheathed), and in other species, they lose it (unsheathed). While taking a blood meal, bloodsucking insects such as mosquitoes, midges, blackflies, and tabanid flies ingest microfilariae. In appropriate blood-feeding insects, microfilariae undergo a period of development and become infective third stage filariform larvae. When the infected insect takes another blood meal, the larvae escape from the proboscis and enter the skin through the bite wound. The larvae then require between 8 and 12 months to become mature adults.

Different species of the following genera of mosquitoes are vectors of *W. bancrofti* filariasis depending on geographical distribution. Among them are: *Culex* (*C. annulirostris*, *C. bitaeniorhynchus*, *C. quinquefasciatus*, and *C. pipiens*); *Anopheles* (*A. arabinensis*, *A. bancroftii*, *A. farauti*, *A. funestus*, *A. gambiae*, *A. koliensis*, *A. melas*, *A. merus*, *A. punctulatus* and *A. wellcomei*); *Aedes* (*A. aegypti*, *A. aquasalis*, *A. bellator*, *A. cooki*, *A. darlingi*, *A. kochi*, *A. polynesiensis*, *A. pseudoscutellaris*, *A. rotumae*, *A. scapularis*, and *A. vigilax*); *Mansonia* (*M. pseudotitillans*, *M. uniformis*); *Coquillettidia* (*C. juxtamansonia*). Refer to figure 3–5 for the following references. During a blood meal, an infected mosquito introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound. ❶ They develop in adults that commonly reside in the lymphatics ❷. The female worms measure 80 to 100 mm in length and 0.24 to 0.30 mm in diameter, while the males measure about 40 mm by .1 mm. Adults produce microfilariae measuring 244 to 296 µm by 7.5 to 10 µm, which are sheathed and have nocturnal periodicity, except the South Pacific microfilariae which have the absence of marked periodicity. The microfilariae migrate into lymph and blood channels moving actively through lymph and blood ❸. A mosquito ingests the microfilariae during a blood meal ❹. After ingestion, the microfilariae lose their sheaths and some of them work their way through the wall of the proventriculus and cardiac portion of the mosquito's midgut and reach the thoracic muscles ❺. There the microfilariae develop into first-stage larvae ❻ and subsequently into third-stage infective larvae ❼. The third-stage infective larvae migrate through the hemocoel to the mosquito's proboscis ❽ and can infect another human when the mosquito takes a blood meal ❶.

### Epidemiology

*W. bancrofti* is endemic in the tropical and subtropical regions of the world, including Latin America, Africa, Turkey, southern Italy, Serbia and Montenegro, Hungary, Asia, and the Pacific Islands. It is common in urban areas with inadequate sanitation favoring breeding of vector mosquitoes. In most areas where the worm is widespread, relatively few individuals are infected. Mosquitoes are the



arthropod vector for *W. bancrofti*. Of the many different species of mosquitoes that can act as intermediate hosts, the most important are *Culex* and *Aedes* species, which prefer human blood and live near human habitations.

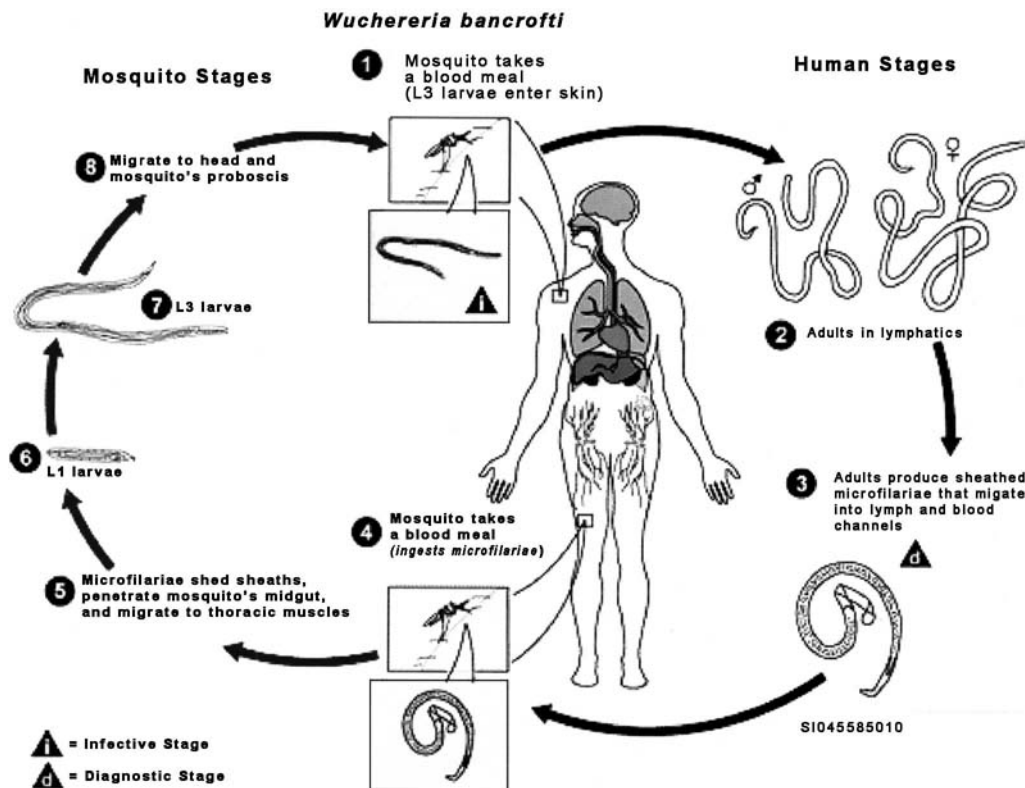


Figure 3-5. Life cycle of *Wuchereria bancrofti*.

### Pathology and clinical manifestations

The adults live in the lymph nodes and lymphatic vessels usually in the groin and external genitalia. The microfilariae commonly circulate, in peripheral blood, only at night, which is called *nocturnal periodicity*. Periodicity is a well-defined circadian rhythm in which certain species circulate in the blood. Strains of the parasite from the South Pacific islands demonstrate *diurnal periodicity* (circulating in the blood during the day) or practically no periodicity (circulate both day and night). During diurnal periodicity, the greatest numbers of parasites appear in the peripheral blood between 12:00 a.m. and 8:00 p.m. Although these strains are morphologically identical to the nocturnal strains, they are sometimes classified by some parasitologists as a separate species designated as *W. pacifica*. In the early stages of infections, there are periods of fever, pain, and some swelling of the lymph nodes and lymph channels. In chronic cases, the lymph vessels become obstructed, and extensive growth of connective tissue develops in the groin, external genitalia, and legs, producing disfigurement (elephantiasis). However, only a small percentage of acute cases continue to the chronic stage of elephantiasis.

### Laboratory diagnosis

The demonstration of adult worms or microfilariae is the only definitive proof of filariasis. *W. bancrofti* is a small thread-like worm. The adult males measure about 40 mm long by 0.1 mm wide, while the females are about 80 to 100 mm long by 0.25 mm wide. In stained blood films, the microfilariae measure 244 to 296  $\mu\text{m}$  long by 7.5 to 10.0  $\mu\text{m}$  wide, they are sheathed, and curve smoothly. The column nuclei are dispersed throughout, they have a short headspace, and the tail tapers to a point and is devoid of nuclei.

**Demonstration of microfilariae**

Techniques for demonstrating microfilariae of *W. bancrofti* in blood specimens also apply to other filariae whose microfilariae circulate in the blood. A careful search for microfilariae should always be made when filariasis is suspected, and examinations should be repeated at intervals. Microfilariae of *W. bancrofti* may be found in preparations of blood or aspirated contents of lymph nodes, hydroceles, or in chylous urine. In areas where *W. bancrofti* demonstrates nocturnal periodicity, microfilariae are most readily detected in blood taken between the hours of 10:00 p.m. and 2:00 a.m. In the South Pacific islands, the nonperiodic microfilariae may be slightly more numerous in the daytime.

**Procedure**

In well-established infections, microfilariae may be seen microscopically, thrashing about in a drop of fresh blood. A finger or earlobe-stick should be performed because the microfilariae concentrate in the peripheral capillaries. However, venous blood can also be used. Mix one drop of blood with one or two drops of physiological saline evenly on a glass slide; spread the preparation and coverslip. It is recommended that multiple slides be made. Use the low power objective for scanning the slide. Wet preparations are good to use for screening purposes. It is always best, however, to examine thick and thin blood smears in suspected cases of filariasis. These smears should be stained with either Giemsa or Delafield's hematoxylin stain and examined under the oil immersion objective. It may be necessary, particularly in new infections, to use a concentration method to detect microfilariae. The Knott technique is most commonly employed, which is a very simple and effective procedure. The technique consists of the following steps:

1. Collect a tube of citrated blood through venipuncture.
2. Add 1 ml of blood to 10 ml of 2 percent formalin.
3. Mix in a 15 ml centrifuge tube.
4. Centrifuge for 5 minutes at 2,000 rpm.
5. Decant the supernatant.
6. Prepare a direct wet mount or stained slides from the sediment and examine for microfilariae.

Stain the sediment with the stains mentioned previously and examine the smears in the same way you would a peripheral blood thin smear. A new concentration technique involves the use of polycarbonate filters (Nuclepore) for trapping microfilariae. The procedure includes lysing the red blood cells and filtering through 3- to 5- $\mu$ m-pore-size filters. The filters may be examined directly on a microscope slide because they are transparent when wet.

**Biopsies**

Verification of the clinical diagnosis even in the absence of microfilariae in the blood can be accomplished by the demonstration of adult worms in lymph node biopsies. Biopsies are not recommended as a routine diagnostic procedure. In the event that biopsies are taken, a pathologist or parasitologist should study them.

**Other studies**

Other laboratory studies are sometimes helpful in filariasis. Leukocytosis and eosinophilia may be present, but they are not specific. Immunological tests of all kinds have been employed, but skin tests and complement-fixation tests have received the most attention. The antigens usually employed are derived from *Dirofilaria immitis*, a filarial that occurs in dogs (heartworm). The tests are not species specific, and the techniques have not been standardized; however, results of these tests may be helpful in conjunction with other findings. They are especially useful when working with large groups of patients, as in surveys. They require very critical evaluation in individual cases.

**Miscellaneous microfilariae**

Now we'll briefly discuss the other microfilariae that you may encounter in the laboratory.

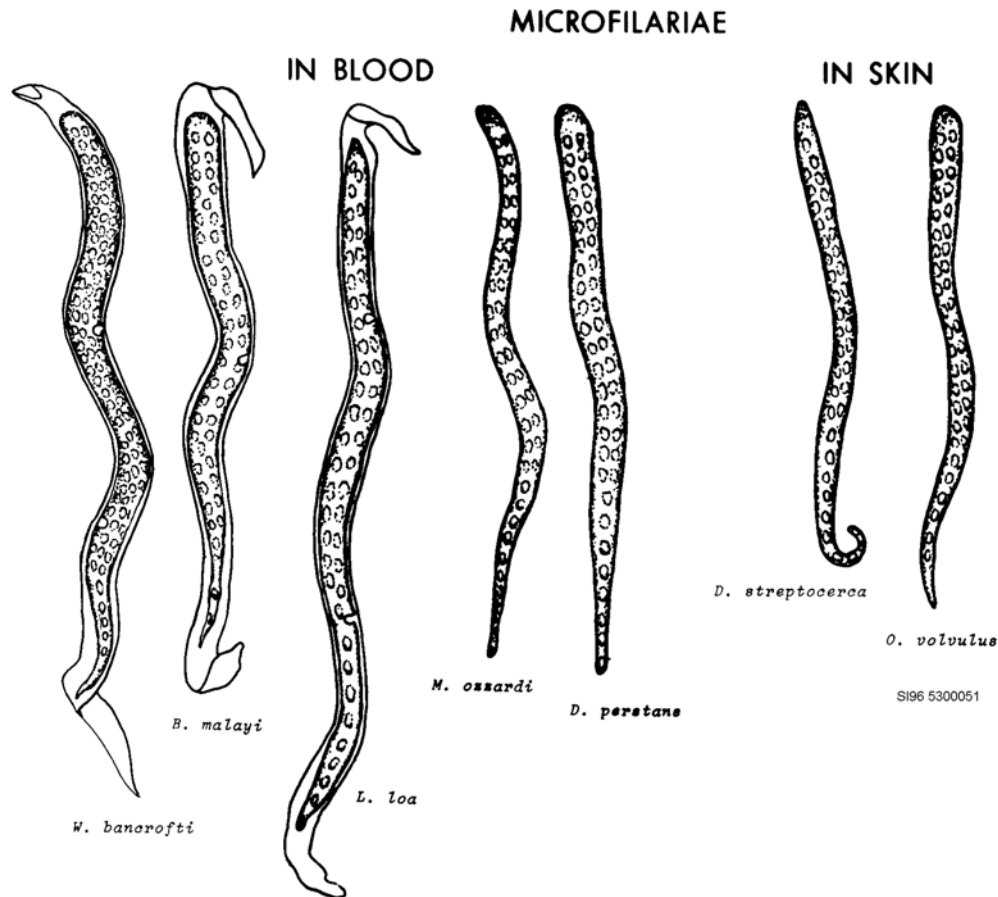


Figure 3-6. Comparison of the microfilariae.

### ***Brugia malayi***

*B. malayi* (fig. 3-7), is found in Asia and the Indian subcontinent and is a mosquito-borne filaria. *Brugia malayi* occurs naturally in cats and monkeys, as well as in humans. The adult parasites live in lymph nodes and vessels. They produce the same type of symptoms and diseases as *W. bancrofti* (for example, lymphangitis, lymphadenitis, and obstructive fibrosis). *B. malayi* adults and microfilariae are very similar to *W. bancrofti*, but they differ sufficiently to be assigned to a different genus. Adult *B. malayi* are about half as large as *W. bancrofti*. The microfilariae of *B. malayi* are sheathed with a tapered tail that has a constriction that separates the column of nuclei from the last nucleus (subterminal and terminal) at the end of the tail (fig. 3-6). The microfilariae measure 177 to 230  $\mu\text{m}$  long by 5 to 6  $\mu\text{m}$  wide in stained smears. They usually exhibit nocturnal periodicity, just as *W. bancrofti*, but the periodicity does not appear to be as strong in some areas. A third species of lymphatic filaria, *Brugia timori*, is found in the eastern end of the Indonesian archipelago and is similar to *B. malayi*.

The typical vector for *Brugia malayi* filariasis are mosquito species from the genera *Mansonia* and *Aedes*. Refer to figure 3-7 for the following references. During a blood meal, an infected mosquito introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound ①. They develop into adults that commonly reside in the lymphatics ②. The adult worms resemble those of *Wuchereria bancrofti* but are smaller. Female worms measure 43 to 55 mm in length by 130 to 170  $\mu\text{m}$  in width, and males measure 13 to 23 mm in length by 70 to 80  $\mu\text{m}$  in width. Adults produce microfilariae, measuring 177 to 230  $\mu\text{m}$  in length and 5 to 7  $\mu\text{m}$  in width, which are sheathed and have nocturnal periodicity. The microfilariae migrate into lymph and enter the blood stream reaching the peripheral blood ③. A mosquito ingests the microfilariae during a blood meal ④.

After ingestion, the microfilariae lose their sheaths and work their way through the wall of the proventriculus and cardiac portion of the midgut to reach the thoracic muscles **5**. There the microfilariae develop into first-stage larvae **6** and subsequently into third-stage larvae **7**. The third-stage larvae migrate through the hemocoel to the mosquito's proboscis **8** and can infect another human when the mosquito takes a blood meal **1**.

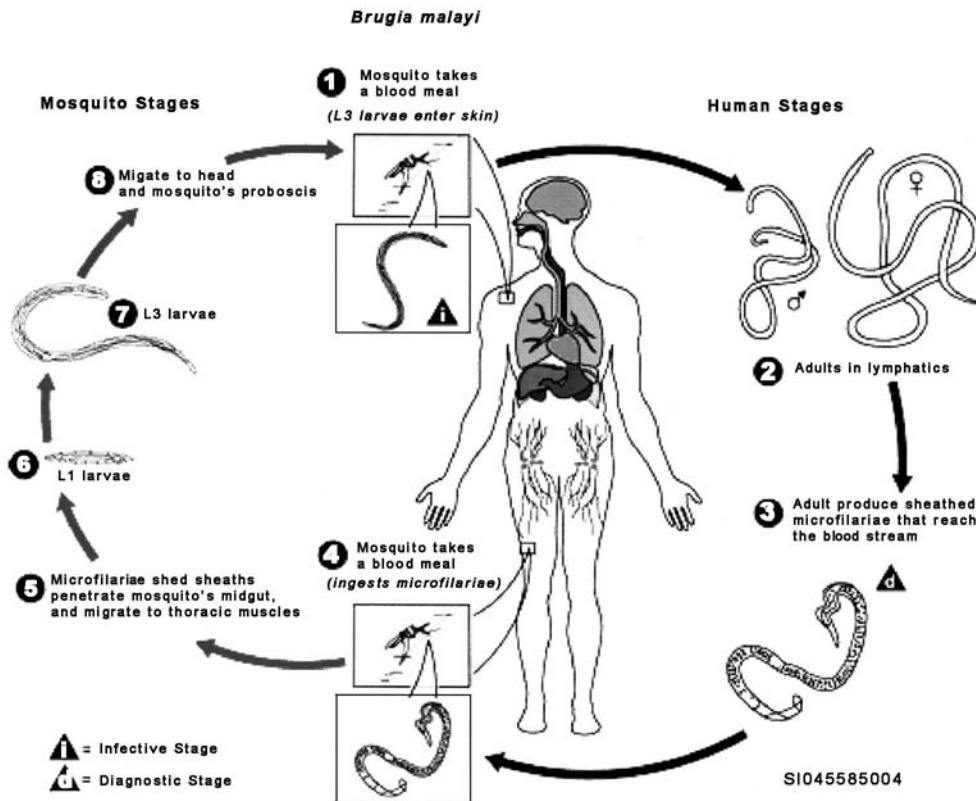


Figure 3-7. Life cycle of *Brugia malayi*.

### *Loa loa*

*Loa loa* is commonly known as the *eye worm* or *missionary worm*. It is found in Central and West Africa. The principal endemic areas lie on the coastal plains from Sierra Leone to Angola and along the watersheds of main rivers. They are medium-sized worms (males are 30 to 35 mm long, while females are 50 to 70 mm long) that inhabit subcutaneous tissues of humans. As the worms migrate around in the subcutaneous tissues, "Calabar swellings" (transient inflammatory reaction) appear and remain for 2 to 3 days. The painful swellings are believed to be an allergic response to the sudden release of toxic metabolites from the adult worms. On occasion, adult worms migrate across the bridge of the nose or beneath the conjunctiva of the eye. The parasite causes no serious damage to the host, but it is rather disturbing for a worm to migrate across the eye. Tabanid flies of the genus *Chrysops* are the intermediate host of *Loa loa*. They usually bite between dawn and dusk—the same time that the microfilariae of *Loa loa* are found in the peripheral circulation. Diagnosis is made by the presence of the microfilariae in the blood or the surgical removal of the adult worm from the conjunctivae. The appearance of microfilariae in the peripheral circulation only during the daytime is called *diurnal periodicity*. The microfilariae, as shown in figure 3-6, are sheathed and have nuclei that extend, without interruption, into the tip of the tail. The sheath of the microfilaria does not stain with Giemsa stain. They are 231 to 250  $\mu\text{m}$  long by 6.0 to 8.5  $\mu\text{m}$  wide. When you are unable to find microfilariae in suspected cases, the patient should be skin-tested with a filarial antigen. An immediate positive reaction will usually occur in people who have the parasite.

The vector for *Loa loa* filariasis are flies from two species of the genus *Chrysops*, *C. silacea* and *C. dimidiata*. Refer to figure 3–8 for the following references. During a blood meal, an infected fly (genus *Chrysops*, day-biting flies) introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound ①. The larvae develop into adults that commonly reside in subcutaneous tissue ②. The female worms measure 40 to 70 mm in length and 0.5 mm in diameter, while the males measure 30 to 34 mm in length and 0.35 to 0.43 mm in diameter. Adults produce microfilariae measuring 250 to 300  $\mu\text{m}$  by 6 to 8  $\mu\text{m}$ , which are sheathed and have diurnal periodicity. Microfilariae have been recovered from spinal fluids, urine, and sputum. During the day they are found in peripheral blood, however during the noncirculation phase, they are found in the lungs ③. The fly ingests microfilariae during a blood meal ④. After ingestion, the microfilariae lose their sheaths and migrate from the fly's midgut through the hemocoel to the thoracic muscles of the arthropod ⑤. There the microfilariae develop into first-stage larvae ⑥ and subsequently into third-stage infective larvae ⑦. The third-stage infective larvae migrate to the fly's proboscis ⑧ and can infect another human when the fly takes a blood meal ①.

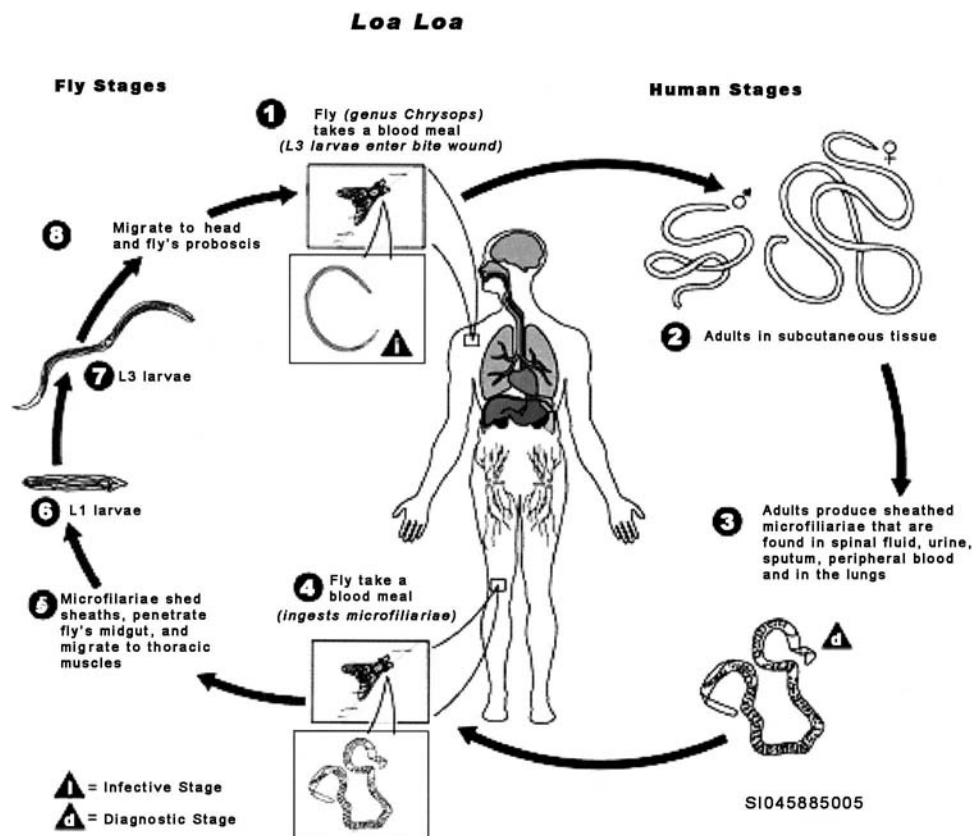


Figure 3–8. Life cycle of *Loa loa*.

### *Mansonella perstans*

This parasite, as well as *M. streptocerca*, was previously allocated to various genera (*Dipetalonema*, *Acanthocheilone*, and *Tetrapietalonema*), but has recently been assigned to the genus *Mansonella*. *M. perstans* is quite common in the tropical regions of western and central Africa, certain Caribbean Islands, and northern South America. It is believed that the adult worm lives in the abdominal cavity and the mesenteries of the human host, while the microfilaria circulate in the blood. Apparently, it is a harmless parasite that lives in the body cavities. Biting midges belonging to the genus *Culicoides* serve as intermediate hosts. You will have to differentiate them from the microfilariae of the more harmful species. The microfilariae are unsheathed, with a blunt tail filled with nuclei, and measures approximately 203  $\mu\text{m}$  long by 4 to 5  $\mu\text{m}$  wide.

Refer to figure 3–9 for references in this paragraph. During a blood meal, an infected midge (genus *Culicoides*) introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound ❶. They develop into adults that reside in body cavities, most commonly the peritoneal cavity or pleural cavity, but less frequently in the pericardium ❷. The size range for female worms is 70 to 80 mm in length and 120  $\mu\text{m}$  in diameter, and the males measure approximately 45 mm by 60  $\mu\text{m}$ . Adults produce unsheathed and subperiodic microfilariae, measuring 200 by 4.5  $\mu\text{m}$  that reach the blood stream ❸. A midge ingests microfilariae during a blood meal ❹. After ingestion, the microfilariae migrate from the midge's midgut through the hemocoel to the thoracic muscles of the arthropod ❺. There the microfilariae develop into first-stage larvae ❻ and subsequently into third-stage infective larvae ❼. The third-stage infective larvae migrate to the midge's proboscis ❽ and can infect another human when the midge takes a blood meal ❶.

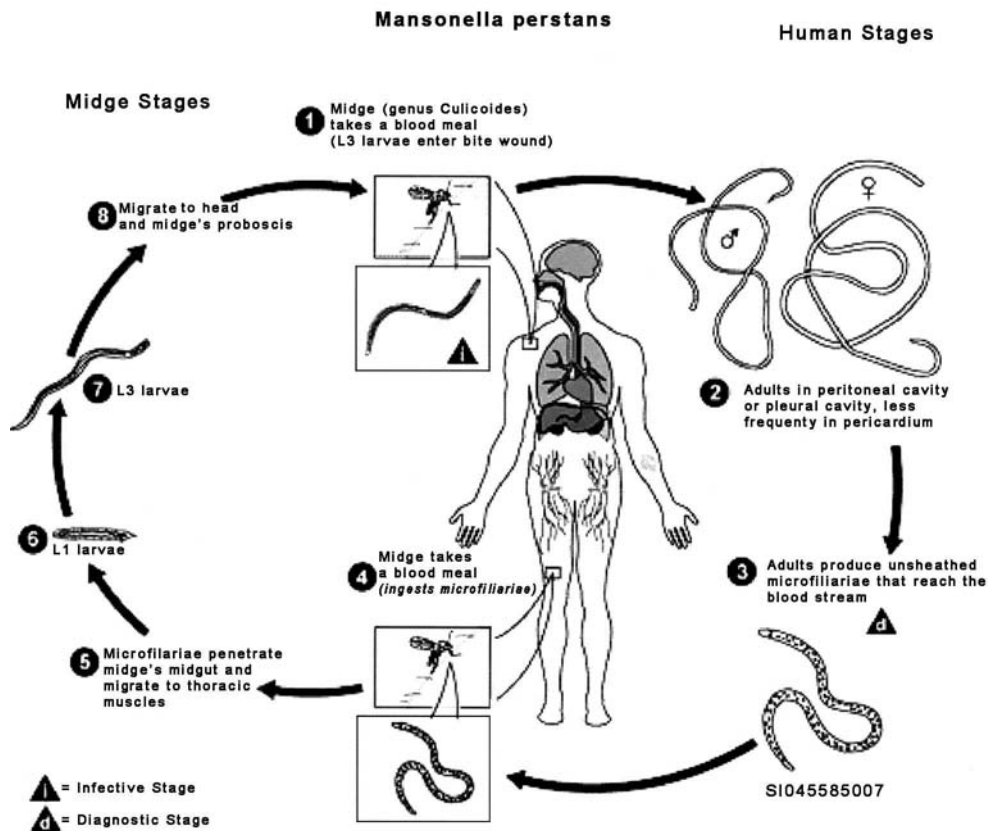


Figure 3–9. Life cycle of *Mansonella perstans*.

### *Mansonella ozzardi*

*Mansonella ozzardi* occurs only in Central and South America, Mexico, Caribbean Islands, and the West Indies. Species of the genus *Culicoides* (midges) and, possibly, species of the genus *Simulium* (black flies) serve as intermediate hosts. The adult worms live in subcutaneous tissue and the microfilariae circulate in the blood at all hours day and night. The microfilariae are small (about 224  $\mu\text{m}$  long by 4 to 5  $\mu\text{m}$  wide), unsheathed, and the long attenuated tail is devoid of nuclei. They apparently cause no harm to the host.

Refer to figure 3-10 for references. During a blood meal, an infected arthropod (midges, genus *Culicoides*, or blackflies, genus *Simulium*) introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound ❶. They develop into adults that commonly reside in subcutaneous tissues ❷. Adult worms are rarely found in humans. The size range for females worms is 65 to 81 mm in length and 0.21 to 0.25 mm in diameter but unknown for males.



Adult worms recovered from experimentally infected Patas monkeys measured 24 to 28 mm in length and 70 to 80  $\mu\text{m}$  in diameter (males) and 32 to 62 mm in length and .130 to .160 mm in diameter (females). Adults produce unsheathed and non-periodic microfilariae that reach the blood stream **3**. The arthropod ingests microfilariae during a blood meal **4**. After ingestion, the microfilariae migrate from the arthropod's midgut through the hemocoel to the thoracic muscles **5**. There the microfilariae develop into first-stage larvae **6** and subsequently into third-stage infective larvae **7**. The third-stage infective larvae migrate to arthropod's proboscis **8** and can infect another human when the arthropod takes a blood meal **1**.

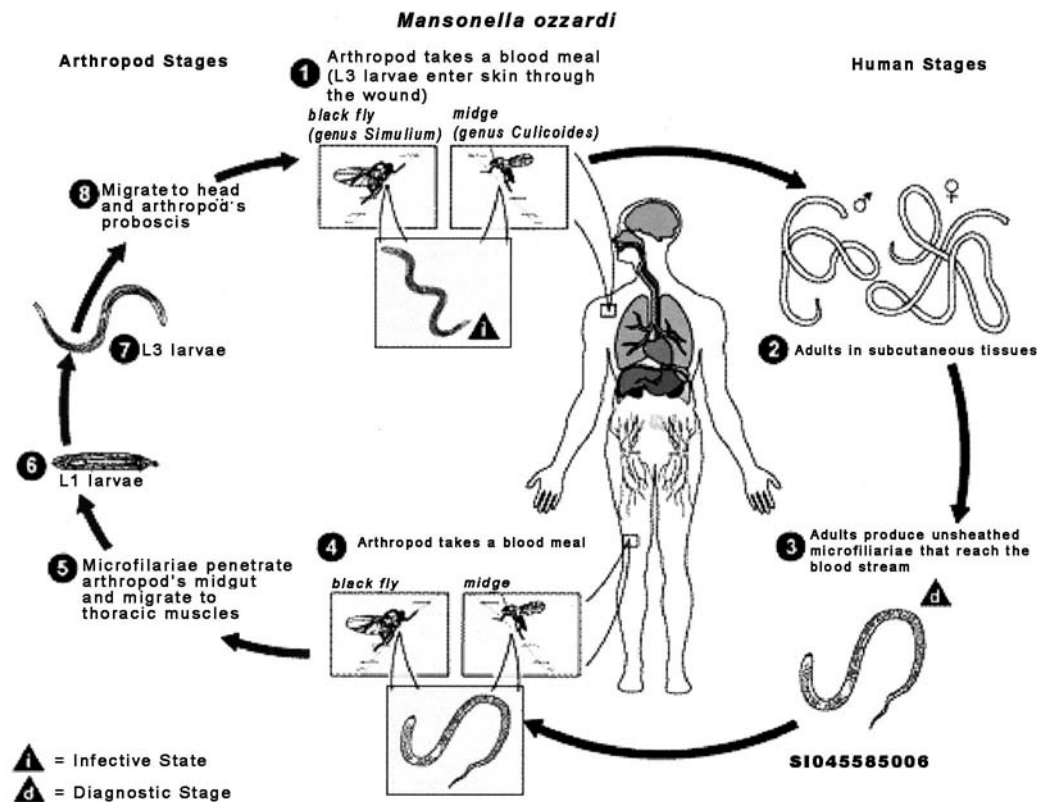


Figure 3–10. Life cycle of *Mansonella ozzardi*.

### *Mansonella streptocerca*

*M. streptocerca* is common in the rain forest belt of Africa and is a skin-dwelling filaria. Species of *Culicoides* serve as intermediate hosts of this parasite. Adult worms live in dermal layers of the skin with the microfilariae. They are similar to the microfilariae of *O. volvulus*. The microfilariae of *M. streptocerca* are unsheathed, long and slender (210  $\mu\text{m}$  by 5 to 6  $\mu\text{m}$ ), and have a characteristic “crooked” tail with nuclei present. They are found most frequently in skin snips, but you must make the snip deep enough to include dermal tissue. Most infected persons are asymptomatic, but they may have some cutaneous edema and disfigurement.

Refer to figure 3–11 for the following references. During a blood meal, an infected midge (genus *Culicoides*) introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound **1**. They develop into adults that reside in the dermis, most commonly less than 1 mm from the skin surface **2**. The females measure approximately 27 mm in length. Their diameter is 50  $\mu\text{m}$  at the level of the vulva (anteriorly) and ovaries (near the posterior end), and up to 85  $\mu\text{m}$  at the mid-body. Males measure 50  $\mu\text{m}$  in diameter. Adults produce unsheathed and non-periodic microfilariae, measuring 180 to 240  $\mu\text{m}$  by 3 to 5  $\mu\text{m}$ , which reside in the skin but can also reach the peripheral blood **3**. A midge ingests the microfilariae during a blood meal **4**. After ingestion, the microfilariae migrate from the midge's midgut through the hemocoel to the thoracic muscles **5**.

There the microfilariae develop into first-stage larvae <sup>6</sup> and subsequently into third-stage larvae <sup>7</sup>. The third-stage larvae migrate to the midge's proboscis <sup>8</sup> and can infect another human when the midge takes another blood meal <sup>1</sup>.

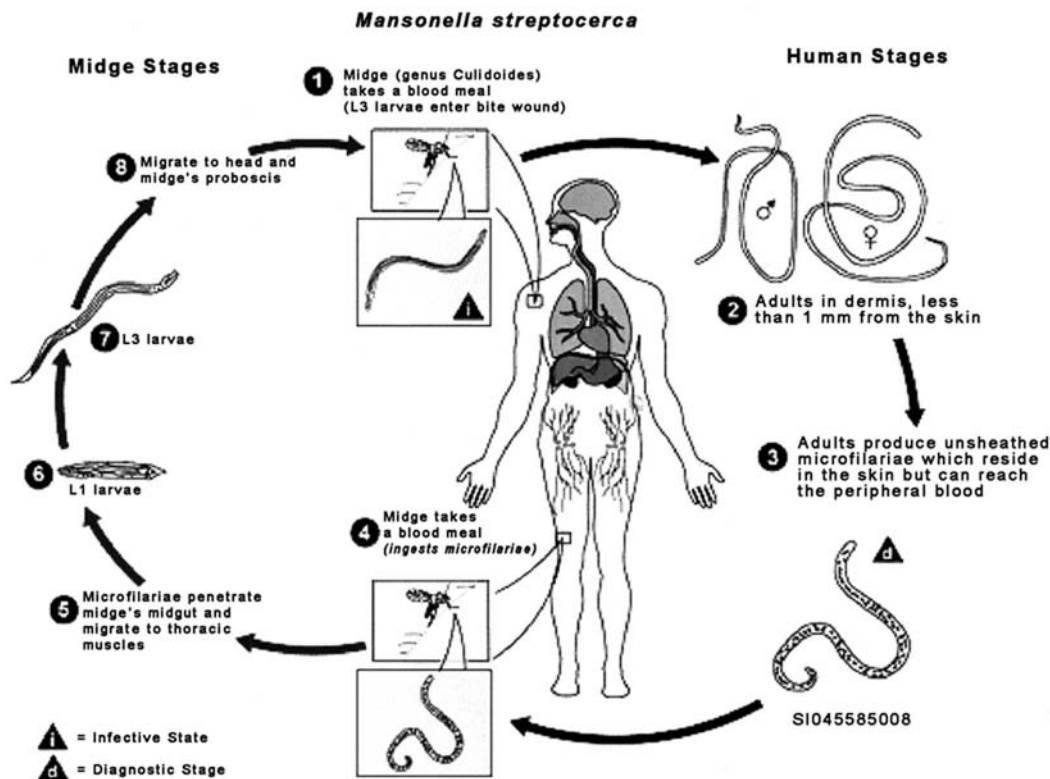


Figure 3-11. Life cycle of *Mansonella streptocerca*.

### *Onchocerca volvulus*

*Onchocerca volvulus* has a very irregular distribution in both hemispheres. The *principle* endemic foci in Africa are inland, along river courses; whereas in Central and South America, the disease is confined to coffee plantations at elevations of 1,000 to 3,500 feet. The adult worms are quite long and slender (males are 19 to 42 mm long by 0.13 to 0.21 mm wide and females are 335 to 500 mm long by 0.27 to 0.40 mm wide). They usually live in pairs in subcutaneous nodules. The nodules can occur on any part of the body. In Africa, the nodules frequently occur over any bony prominences of the body; whereas in Central and South America, they are found more frequently on the head or scalp. The microfilariae are unsheathed and approximately 309  $\mu$ m long by 5 to 9  $\mu$ m wide. The tail is tapered, usually bent, without nuclei. The microfilariae of *Onchocerca* remain in the superficial lymphatic spaces and connective tissues of the skin after they escape from the nodules. They rarely, if ever, get into the bloodstream. The insect intermediate hosts belong to the genus *Simulium*, commonly known as *black flies*. Members of the genus *Simulium* are bloodsuckers, but they have chewing mouthparts. To take a blood meal, they chew a hole in the skin to the capillary bed. Then they lap up the blood and, at the same time, the tissue juices that contain the microfilariae of *O. volvulus*. The nodules of *Onchocerca volvulus* cause little or no inconvenience. As the microfilariae migrate about through the skin, sensitization reactions occur. In addition to itching, the skin becomes dry, wrinkled, rough and shiny. Eventually, the skin thickens and loses its elasticity. In some areas, among people who have *Onchocerca volvulus* infections there is a high incidence of blindness. Blindness is a result of the damage caused by microfilariae migrating through the eye. The microfilariae do not enter the bloodstream; therefore, you cannot find them by examining blood smears, not even with concentration methods. Since the microfilariae are in the skin, the examination of skin snips is the most practical diagnostic measure. Remove the skin with a razor blade, tease the



specimen apart in a drop of saline or water, and examine it microscopically for microfilariae. Excised nodules should be examined for microfilariae and adult worms.

Refer to figure 3-12 for the following references. During a blood meal, an infected blackfly (genus *Simulium*) introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound **1**. In subcutaneous tissues the larvae **2** develop into adult filariae, which commonly reside in nodules in subcutaneous connective tissues **3**. Adults can live in the nodules for approximately 15 years. Some nodules may contain numerous male and female worms. Females measure 33 to 50 cm in length and 270 to 400  $\mu\text{m}$  in diameter, while males measure 19 to 42 mm by 130 to 210  $\mu\text{m}$ . In the subcutaneous nodules, the female worms are capable of producing microfilariae for approximately 9 years. The microfilariae, measuring 220 to 360  $\mu\text{m}$  by 5 to 9  $\mu\text{m}$  and unsheathed, have a life span that may reach 2 years. They are occasionally found in peripheral blood, urine, and sputum but are typically found in the skin and in the lymphatics of connective tissues **4**. A blackfly ingests the microfilariae during a blood meal **5**. After ingestion, the microfilariae migrate from the blackfly's midgut through the hemocoel to the thoracic muscles **6**. There the microfilariae develop into first-stage larvae **7** and subsequently into third-stage infective larvae **8**. The third-stage infective larvae migrate to the blackfly's proboscis **9** and can infect another human when the fly takes a blood meal **1**.

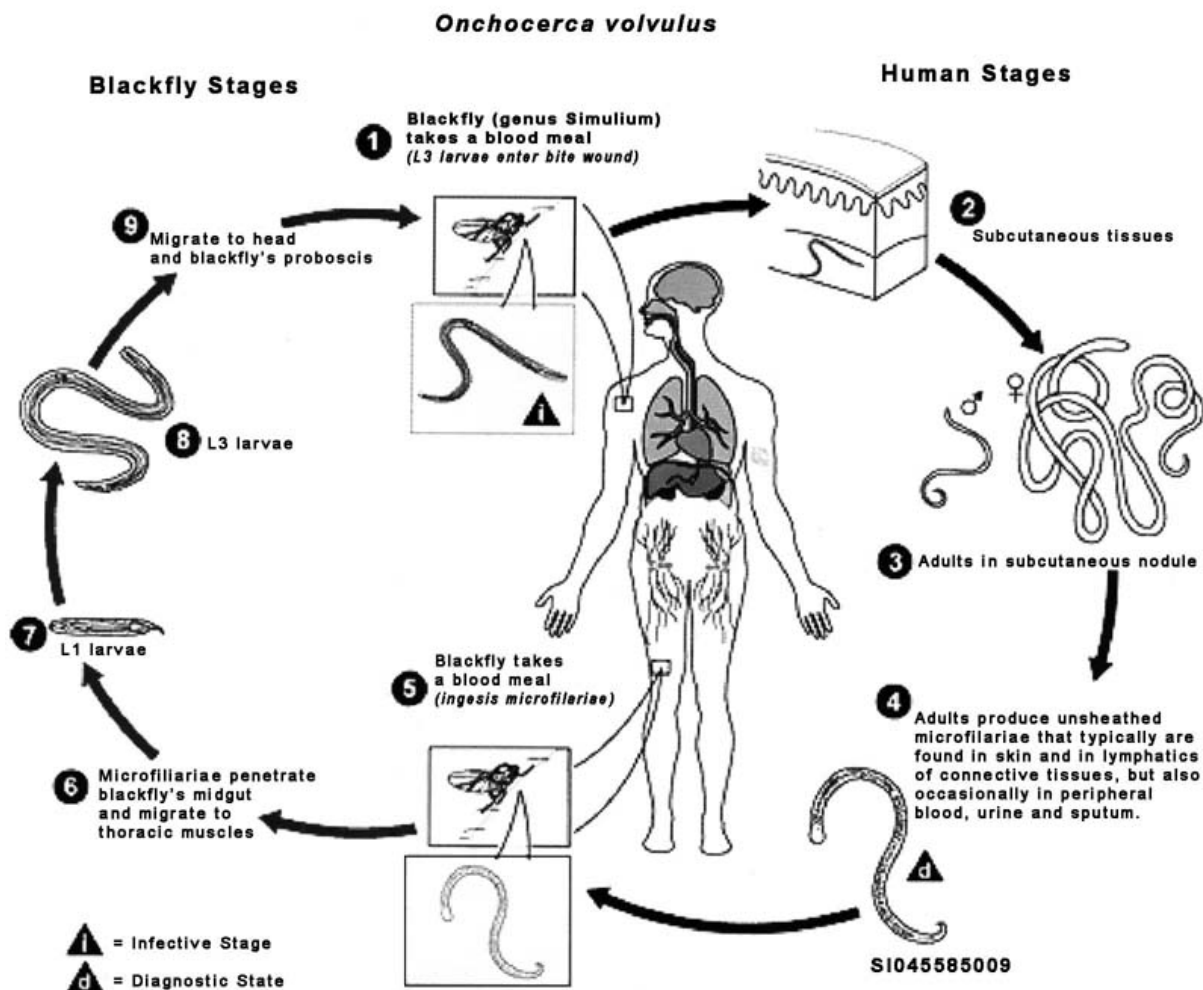


Figure 3-12. Life cycle of *Onchocerca volvulus*.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 619. Tissue Helminths

1. What animals are the reservoirs for human infection of *Trichinella*?
2. How are the larvae distributed to all parts of the body?
3. After the young larvae leave the capillaries, what type of muscle do they invade?
4. Where are the most frequently affected muscles located?
5. At what stage of development does the larvae become infective?
6. When does calcification of the encapsulated larvae begin?
7. What symptoms does a patient exhibit as the young worms excyst and migrate into the intestinal mucosa?
8. What is the mortality rate if the central nervous system is involved and is left untreated?
9. What technique can be used for viewing the encapsulated larvae in tissue?
10. What is the standard serological method for diagnosis of *T. spiralis*?
11. What is the natural definitive host of *Gnathostoma*?
12. What is the intermediate host of *E. granulosus*? Accidental host?

13. How does the infection caused by *E. granulosus* occur?

14. Where do the majority of hydatid cysts appear?

## 620. Tissue Helminths

1. Match each column B item with the correct statement in column A. Place the letter of the proper column B item beside the number of the column A item most nearly describing it. Each column B element may be used once, more than once, or not at all.

### Column A

- \_\_\_\_ (1) Is endemic in the tropical and subtropical regions of the world, including Latin America, Africa, Turkey, southern Italy, Serbia and Montenegro, Hungary, Asia, and the Pacific Islands.
- \_\_\_\_ (2) The adult worms live in lymph nodes and lymphatic vessels usually in the groin and external genitalia.
- \_\_\_\_ (3) The microfilariae are unsheathed, long and slender, and have a “crooked” tail.
- \_\_\_\_ (4) Is found in Southeast Asia; this range extends from Ceylon northward into India, and from Indonesia and Borneo through Malaya and Thailand to South Korea.
- \_\_\_\_ (5) Microfilariae may be found in preparations of blood or aspirated contents of lymph nodes, hydroceles, or chylous urine.
- \_\_\_\_ (6) In this technique, 1 ml of blood is withdrawn from the vein and diluted in 10 ml of 2% formalin.
- \_\_\_\_ (7) The antigens are derived from this filariid, which occurs in dogs, and used in the complement fixation.
- \_\_\_\_ (8) The microfilariae have a subterminal and terminal at the end of the tail.
- \_\_\_\_ (9) Is commonly known as the eye worm or missionary worm.
- \_\_\_\_ (10) The microfilariae appear in the peripheral circulation mainly during the daytime.
- \_\_\_\_ (11) They usually live in pairs in subcutaneous nodules.
- \_\_\_\_ (12) They remain in the superficial lymphatic spaces and connective tissue of the skin after they escape from the nodules.
- \_\_\_\_ (13) The examination of skin snips is the most practical diagnostic measure for this microfilariae.
- \_\_\_\_ (14) Is apparently a harmless parasite that lives in the body cavities, and biting midges belonging to the genus *Culicoides* serve as intermediate hosts.
- \_\_\_\_ (15) Adult worms live in dermal layers of the skin with the microfilariae and are similar to the microfilariae of *O. volvulus*.
- \_\_\_\_ (16) Occurs only in both hemispheres.
- \_\_\_\_ (17) Microfilariae commonly circulate in the peripheral blood only at night (nocturnal periodicity).

### Column B

- a. *Wuchereria bancrofti*.
- b. *Dirofilaria immitis*.
- c. *Mansonella ozzardi*.
- d. *Mansonella perstans*.
- e. *Loa loa*.
- f. *Onchocerca volvulus*.
- g. *Brugia malayi*.
- h. *Mansonella streptocerca*.
- i. Knott’s technique.
- j. Baermann’s technique.

## Answers to Self-Test Questions

### 619

1. The pig, bear, walrus, horse meat, or meat from other carnivores that contain viable infective larvae.
2. By the intestinal lymphatic system or mesenteric venules.
3. Voluntary (striated).
4. In the diaphragm, larynx, tongue, jaws, neck, ribs, biceps, gastrocnemius, and deltoids.
5. After they become coiled and encapsulation begins.
6. After about 8 weeks.
7. Symptoms of gastroenteritis or food poisoning.
8. The mortality rate for these patients may reach 50%.
9. Compress a portion of the tissue between two glass slides and examine microscopically.
10. Bentonite flocculation test.
11. Pigs, cats, dogs, and wild animals.
12. Sheep and cattle are considered intermediate hosts and humans are accidental intermediate host.
13. Infection occurs by ingesting the typical taeniid eggs excreted in the feces of infected dogs (definitive hosts).
14. The majority of hydatid cysts appear in the liver but can appear in the lungs and other organs where they are usually asymptomatic.

### 620

1. (1) a.  
(2) a.  
(3) a.  
(4) h.  
(5) a.  
(6) i.  
(7) b.  
(8) g.  
(9) e.  
(10) e.  
(11) f.  
(12) f.  
(13) f.  
(14) d.  
(15) h.  
(16) c.  
(17) a, g.

**Do the unit review exercises before going to the next unit.**

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## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter.

45. (619) The animal serving as the **principal** reservoir for human *Trichinella spiralis* infection in humans is the
- a. cat.
  - b. cow.
  - c. dog.
  - d. pig.
46. (619) *Echinococcus* primarily infects the
- a. lungs.
  - b. liver.
  - c. marrow.
  - d. bloodstream.
47. (620) In areas where *Wuchereria bancrofti* demonstrate nocturnal periodicity, *microfilariae* are detected *most* readily in blood specimens taken
- a. at night.
  - b. during the day.
  - c. in the early morning.
  - d. in the early afternoon.
48. (620) The **only** definitive proof of filariasis, is the demonstration of adult worms or
- a. ova.
  - b. microfilariae.
  - c. encysted larvae.
  - d. filariform larvae.

Please read the unit menu for unit 4 and continue ➔

## **Student Notes**

## Unit 4. Lumen-Dwelling Protazoa

|                                                                     |             |
|---------------------------------------------------------------------|-------------|
| <b>4-1. Amebae .....</b>                                            | <b>4-1</b>  |
| 621. <i>Entamoeba histolytica</i> .....                             | 4-1         |
| <b>4-2. Flagellates and Ciliates.....</b>                           | <b>4-8</b>  |
| 622. <i>Dientamoeba fragilis</i> .....                              | 4-8         |
| 623. <i>Giardia lamblia</i> .....                                   | 4-10        |
| 624. <i>Trichomonas vaginalis</i> .....                             | 4-12        |
| 625. <i>Balantidium coli</i> .....                                  | 4-14        |
| <b>4-3. Coccidia and Microsporidia .....</b>                        | <b>4-19</b> |
| 626. <i>Isospora belli</i> and <i>Cryptosporidium</i> species ..... | 4-19        |

**N**UMEROUS species of the subkingdom *Protozoa* inhabit the various environments of the Earth, including the intestinal tract of humans. In this subkingdom, there are the amebae, flagellates, ciliates, coccidia, and microsporidia. Most of these organisms are harmless; however, a few can cause serious disease in humans and animals. The most common pathogenic protozoa are addressed in this unit. Also, as with the helminths, the life cycle, epidemiology, pathology, and laboratory diagnosis of each organism are reviewed. Hopefully, this approach will give you a well-rounded view of the organism and its impact on the health of those living in endemic areas.

### 4-1. Amebae

The process of finding and accurately identifying cysts and trophozoites of amebae in the routine examination of specimens is one of the most difficult tasks confronting you as a laboratory technician. You must be able to differentiate those of medical importance from those that are harmless. *E. histolytica* is the only confirmed amebic pathogenic to humans.

#### 621. *Entamoeba histolytica*

*Amebiasis* is a very common, sometimes virulent disease in the tropics caused by *E. histolytica*. *E. histolytica*. It can live as a commensal in the lumen of the large intestine, or it can invade the host tissues.

#### Virulence

Several factors are thought to be responsible for determining the degree of virulence expressed by the organism. Individual variation in the pH, bacterial flora, and nutritional factors seem to influence the course of this disease. However, it is known that strains isolated from asymptomatic patients are usually less invasive or noninvasive to laboratory animals. General host defense mechanisms, previous contact with parasite, diet, and the strain of *E. histolytica* can influence the manifestations of infection in the individual. The study of *E. histolytica* zymodemes (isoenzyme patterns) is a useful technique for differentiating between pathogenic and nonpathogenic strains of *E. histolytica*.

#### Life cycle

As illustrated in figure 4-1, amebiasis due to *E. histolytica* begins with the ingestion of a mature metacyst with four nuclei. If a trophozoite is ingested, it disintegrates in the stomach without producing infection. The mature cysts are capable of passing through the acid environment of the stomach unharmed and show no activity during this stage of their passage. On entering the alkaline environment of the small intestine, however, the metacyst stirs within the confines of the cyst wall, which soon weakens, allowing the mature ameba to escape and rapidly divide into eight amebulae. These are swept by peristaltic action into the cecum (the first part of the large intestine). The

metacystic trophozoites then can invade the host tissue, live in the lumen of the large intestine without invasion, or undergo encystation.

### Encystation

The third option—encystation—enables *E. histolytica*'s life cycle to continue and to infect yet another victim. Water is withdrawn from the fecal mass as it moves through the large bowel. As the fecal matter becomes dehydrated, the amebas are stimulated to encyst. Cysts are neither found in the stools of patients with dysentery nor formed by amebas when they invade the tissue of the host. As the trophozoite begins to encyst, it disgorges any undigested food and rounds up into a precyst. Often, a precyst is so rich in glycogen that it will contain a large glycogen vacuole, which may occupy a large portion of its cytoplasm. Chromatoid bars, which are composed of condensed ribonucleic acid RNA, are also formed at this time. They can be short and thick, thin and curved, spherical, or very irregular in shape, but they do not have the splinter-like appearance found in *E. coli*. The precyst then secretes a thin, tough wall around itself for protection from the environment and becomes a cyst. This protective wall enables the cyst to endure the unfavorable environmental conditions it will encounter outside the body. The cyst may be somewhat ovoid or elongate, but it is usually spheroid. It is commonly 10 to 20  $\mu\text{m}$  wide. The rate at which the fecal mass is expelled from the body determines the degree of maturity of the cyst found in the patient's sample. If the sample had time to become a formed mass, the majority of the organisms will be of the typical mature or metacyst, with four nuclei and no glycogen vacuoles or chromatoid bars. If the feces leave the body in a semiformed mass, you are more likely to see precysts or immature cysts with glycogen vacuoles and chromatoid bodies.

### Causal agent

Several protozoan species in the genus *Entamoeba* infect humans, but not all of them are associated with disease. *Entamoeba histolytica* is well recognized as a pathogenic ameba, associated with intestinal and extraintestinal infections. The other species are important because they may be confused with *E. histolytica* in diagnostic investigations.

Refer to figure 4-1 for following references. Cysts are passed in feces ①. Infection by *Entamoeba histolytica* occurs by ingestion of mature cysts ② in fecally contaminated food, water, or hands. Excystation ③ occurs in the small intestine and trophozoites ④ are released, which migrate to the large intestine. The trophozoites multiply by binary fission and produce cysts ⑤, which are passed in the feces ①. Because of the protection conferred by their walls, the cysts can survive days to weeks in the external environment and are responsible for transmission. (Trophozoites can also be passed in diarrheal stools, but are rapidly destroyed once outside the body, and if ingested would not survive exposure to the gastric environment.) In many cases, the trophozoites remain confined to the intestinal lumen (A: noninvasive infection) of individuals who are asymptomatic carriers, passing cysts in their stool. In some patients the trophozoites invade the intestinal mucosa (E: intestinal disease), or through the bloodstream, extraintestinal sites such as the liver, brain, and lungs (C: extraintestinal disease), with resultant pathologic manifestations. It has been established that the invasive and noninvasive forms represent two separate species, respectively *E. histolytica* and *E. dispar*, however not all persons infected with *E. histolytica* will have invasive disease. These two species are morphologically indistinguishable. Transmission can also occur through fecal exposure during sexual contact (in which case not only cysts, but also trophozoites could prove infective).

### Geographic Distribution

The distribution is worldwide, with higher incidence of amebiasis in developing countries. In industrialized countries, risk groups include male homosexuals, travelers and recent immigrants, and institutionalized populations.



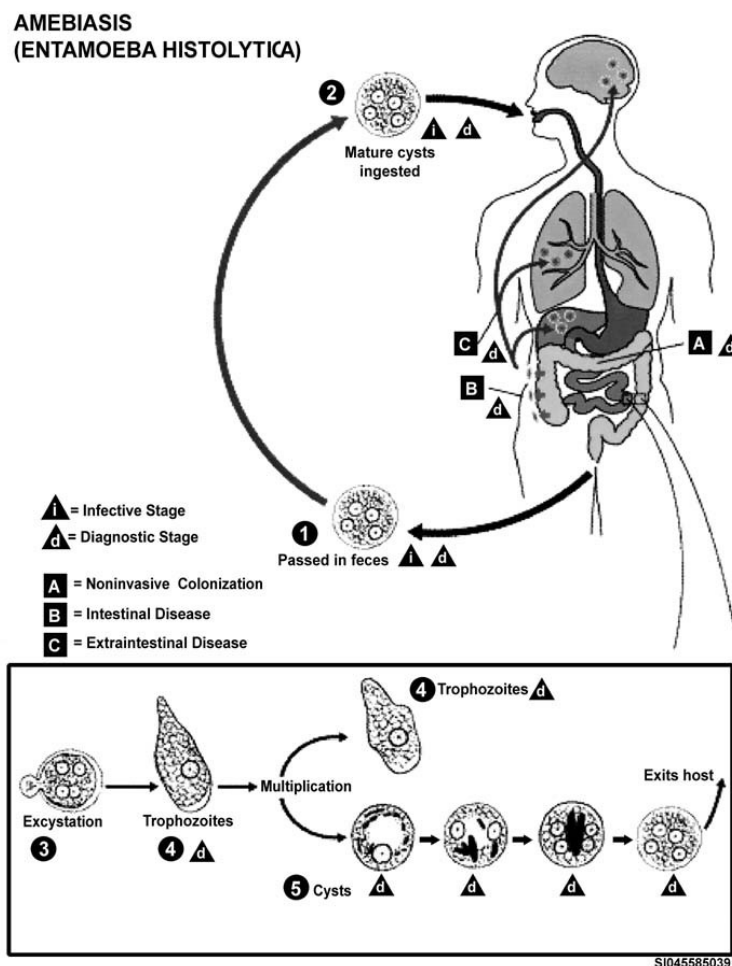


Figure 4-1. Life cycle of *Entamoeba histolytica*.

### Epidemiology

Amebic infections occur worldwide, with an estimated 400 million cases in existence. The prevalence of amebic infections closely parallels the level of sanitation in a region. The better the disposal of human feces, the less likely that amebic infections will occur. Infected individuals may be asymptomatic for long periods, yet excrete large numbers of infectious metacysts. Such cysts can survive for as long as 30 days in a cool, moist atmosphere in an environment having a low bacterial population. At higher temperatures, and in the presence of large numbers of bacteria, the life expectancy of the cysts is reduced significantly.

### Common sources of infection

The most common sources of infection are contaminated food and/or water that contains the infective cyst. In many parts of the world, there is little if any effort to treat water. Even if chlorination is available, if the water is contaminated after treatment was accomplished, the cysts are still infectious since normal levels of chlorine in treated water are not adequate to destroy the cysts. In such cases hyperchlorination, treatment with iodine, or filtration is required to make the water supply safe for human consumption. Food can be contaminated in a number of ways. In poorer areas of the world, where human night soil (feces) is used extensively as organic fertilizer, certain vegetables normally eaten raw can act as a source of infection. In these same areas, flies, cockroaches, and other insects can transmit cysts from improperly disposed feces to food items. The sticky, bristly appendages of these vectors can easily carry cysts from a fresh stool sample to the dinner table, and the habit of the

housefly to vomit and defecate while it feeds has been shown to be an important means of transmission.

### **Pathology and clinical manifestations**

Amebic dysentery caused by *E. histolytica* is a severe, acute disease that manifests with bloody diarrhea and abdominal cramping. Amebic dysentery is not frequently encountered in the U.S., eclipsed by the amebic colitis. The patient with amebic colitis may exhibit some or all of the following symptoms:

- Diarrhea.
- Constipation.
- Abdominal cramping.
- Weight loss.

These symptoms are seen without the blood and mucus seen in amebic dysentery. In the dysentery form, the organism will invade the mucosa often so severely that perforations are created and extraintestinal infection may ensue. The most common form of extraintestinal amebiasis is amebic abscess of the liver, which occurs in approximately 5 percent of symptomatic patients resulting in upper right quadrant abdominal pain. The organisms are found in the stool in less than 50 percent of patients that have liver abscesses. The organism has been known to infect other organs such as the lung and brain, but this is a rare occurrence.

### **Laboratory diagnosis**





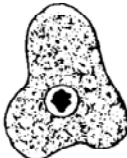
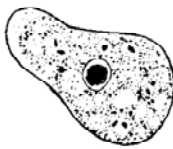







Examination of a series of stool specimens allows diagnosis of the intestinal infection in most cases. If the patient has been given antibiotics or gallbladder dyes, the amebic infection may be suppressed for a period. If there is a strong suspicion of amebiasis and stool examinations are negative, collection of a purged series is recommended. Some laboratories use culture procedures to grow the amebae. Culture is essential if zymodemes are to be determined to see if the strain is pathogenic. Serologic tests are positive in approximately 70 percent of patients with invasive intestinal amebiasis but in only 5% of patients with commensal infection. Aspirated material from liver abscesses can be examined microscopically to detect trophozoites. The last material aspirated is most likely to contain trophozoites and can be examined by direct microscopic examination or permanently stained slides. If tissue is available, sections may show organisms. Culture procedures can be attempted, but since bacteria are not present in the liver abscesses, they (for example, *Clostridium perfringens*) will need to be added to the culture. Serologic tests are positive in over 95 percent of patients with amebic liver abscess.

### **Trophozoites**

The trophozoites of *Entamoeba histolytica* vary from 10 to 60  $\mu\text{m}$ , with the commensal forms usually 15 to 20  $\mu\text{m}$ , and the invasive forms over 20  $\mu\text{m}$  in their greatest dimension, as shown in figure 4-2. In direct wet mounts, the trophozoites show progressive motility, with hyaline pseudopodia that are rapidly formed by sharp demarcation between endoplasm and ectoplasm, but unstained nuclei are not visible. In severe disease, some trophozoites may contain ingested erythrocytes, a feature diagnostic of *E. histolytica* infection. The karyosome is small and centrally located, with fine fibrils attaching it to the nuclear membrane; however, these fibrils are not generally visible. Nuclei vary, and eccentric karyosomes and irregularly distributed peripheral chromatin may be seen. The appearance of amebae may vary, and there is no single characteristic that is *pathognomonic* (specifically distinctive or characteristic of a disease or pathologic condition), except the phagocytosis of erythrocytes, which very rarely occurs with other species. The cytoplasm is finely granular, and in invasive organisms, there are either no inclusions or only erythrocyte inclusions. Noninvasive organisms can, occasionally, contain ingested bacteria. In degenerated organisms, the cytoplasm can become vacuolated, and nuclei can show abnormal chromatin clumping.

# PROTOZOA FOUND IN STOOL SPECIMENS OF HUMANS

9800089 961S

| AMEBAE      |                                                                                       |                                                                                       |                                                                                       |                                                                                      |                                                                                     |                                                                                     |                                                                                   |
|-------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
|             | <i>Entamoeba histolytica</i>                                                          | <i>Entamoeba hartmanni</i>                                                            | <i>Entamoeba coli</i>                                                                 | <i>Entamoeba polecki</i> <sup>1</sup>                                                | <i>Endolimax nana</i>                                                               | <i>Iodamoeba bütschlii</i>                                                          | <i>Dientamoeba fragilis</i> <sup>2</sup>                                          |
| Trophozoite |    |    |    |    |    |    |  |
| Cyst        |  |  |  |  |  |  | No cyst                                                                           |

<sup>1</sup> Rare, probably of animal origin  
<sup>2</sup> Flagellate

Scale: 0 5 10  $\mu$ m

Adapted from Brooke and Melvin, 1964

Figure 4-2. Morphology of protozoan trophozoites and cyst found in human stool specimens.

### Cysts

Cysts of *E. histolytica* are spherical and 10 to 20  $\mu\text{m}$  in diameter, with the usual range being 12 to 15  $\mu\text{m}$ . The precyst stage has a single nucleus and is rounded, but it does have a refractile cyst wall. As it matures, the cyst develops four nuclei, each of which is approximately one-sixth the diameter of the cyst. The nuclei show characteristics similar to those of trophozoite nuclei, but let us stress that nuclear characteristics are not as helpful in differentiation of *Entamoeba* cysts as they are in differentiation of trophozoites. The cytoplasm of the cyst can contain glycogen vacuoles and chromatoid bodies with blunted or rounded ends. The number and size of nuclei and the appearance of chromatoid bodies are good diagnostic criteria for cysts.

### Miscellaneous amebae

The following table gives a quick review of the characteristics of the trophozoites and cysts of *E. histolytica* and the other usually non-pathogenic amebae.

#### In the trophozoite form

This first table presents the differential morphology of protozoa found in stool specimens of human amebae in the trophozoite form.

| Species                      | Trophozoite Size (length) | Motility                                                | Karyosome chromatin                                                                                                               | Cytoplasm                                                                     |
|------------------------------|---------------------------|---------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| <i>Entamoeba histolytica</i> | 12 to 60 $\mu\text{m}$    | Progressive with hyaline, finger-like pseudopods        | Small, discrete, usually centrally located, but occasionally is eccentric                                                         | Finely granular, RBC or bacterial inclusions occasionally                     |
| <i>Entamoeba hartmanni</i>   | 5 to 10 $\mu\text{m}$     | Usually non-progressive                                 | small, discrete often eccentric                                                                                                   | Finely granular, bacteria inclusions                                          |
| <i>Entamoeba coli</i>        | 10 to 35 $\mu\text{m}$    | Sluggish, non-progressive, with blunt pseudopods        | Large, discrete usually eccentric                                                                                                 | Coarse, often vacuolated, bacteria, yeast's, other material inclusions        |
| <i>Entamoeba polecki</i>     | 9 to 18 $\mu\text{m}$     | Usually sluggish, progressive in diarrheic specimens    | Small, discrete, eccentric, occasionally large, diffuse, or irregular                                                             | Coarsely granular, numerous vacuoles, bacteria and yeast inclusions           |
| <i>Endolimax nana</i>        | 5 to 10 $\mu\text{m}$     | Sluggish, usually non-progressive with blunt pseudopods | Large, irregularly shaped, blot-like                                                                                              | Granular, vacuolated, bacteria inclusions                                     |
| <i>Iodamoeba büttchli</i>    | 5 to 20 $\mu\text{m}$     | Sluggish, usually non-progressive                       | Large, usually central, surrounded by refractile, achromatic granules                                                             | Coarsely granular, vacuolated, bacteria, yeast's or other material inclusions |
| <i>Chilomastix mesnili</i>   | 6 to 24 $\mu\text{m}$     | Stiff, rotating movement                                | Centrally located or situated against nuclear membrane, granular either evenly or irregularly distributed on the nuclear membrane | Cytosomal groove                                                              |

***In the cyst form***

This second table presents the differential morphology of protozoa found in stool specimens of human amebae in the cyst form.

| Species                      | Cyst Size<br>(Diameter or length) | Shape                               | Nucleus                   | Cytoplasm                                                                |
|------------------------------|-----------------------------------|-------------------------------------|---------------------------|--------------------------------------------------------------------------|
| <i>Entamoeba histolytica</i> | 10 to 20 $\mu\text{m}$            | Usually spherical                   | 4 in mature cyst          | Chromatoidal bodies present, glycogen diffuse or absent                  |
| <i>Entamoeba hartmanni</i>   | 5 to 10 $\mu\text{m}$             | Usually spherical                   | 4 in mature cyst          | Chromatoidal bodies present, glycogen diffuse or absent                  |
| <i>Entamoeba coli</i>        | 10 to 35 $\mu\text{m}$            | Usually spherical, occasional ovoid | 8 in mature cyst          | Chromatoidal bodies may be present, glycogen diffuse or absent           |
| <i>Entamoeba polecki</i>     | 9 to 18 $\mu\text{m}$             | Spherical or oval                   | 1 rarely 2 in mature cyst | Chromatoidal bodies present, glycogen diffuse                            |
| <i>Endolimax nana</i>        | 5 to 10 $\mu\text{m}$             | Usually oval may be round           | 4 in mature cyst          | Chromatoidal bodies rarely present, glycogen diffuse                     |
| <i>Iodamoeba büttchlii</i>   | 5 to 20 $\mu\text{m}$             | Vary from oval to round             | 1 in mature cyst          | No chromatoidal bodies present, glycogen large compact well-defined mass |
| <i>Chilomastix meslinii</i>  | 5 to 10 $\mu\text{m}$             | Often lemon shaped                  | 1 large                   | Central karyosome without peripheral chromatin                           |

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### Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

**621. *Entamoeba histolytica***

1. What are three factors that are thought to be responsible for determining the degree of virulence expressed by *E. histolytica*?
2. What technique has been useful in differentiating pathogenic and non-pathogenic strains of *E. histolytica*?
3. How are *E. histolytica* infections acquired?
4. Can the ingestion of *E. histolytica* trophozoites cause amebiasis?
5. What life cycle options exist for trophozoites once they are released from the cyst?
6. What stimulates *E. histolytica* trophozoites to encyst?

7. What two cytoplasmic morphological features are precyst likely to display?
8. What is the function of the cyst wall?
9. What determines the degree of maturity of the cyst found in a patient's sample?
10. To what can the prevalence of *E. histolytica* infections be linked?
11. How long can a cyst of *E. histolytica* survive under favorable environmental conditions?
12. What is the more common condition caused by *E. histolytica* that occurs in the United States?
13. What is the most common form of extraintestinal amebiasis?
14. How are most liver abscesses due to *E. histolytica* diagnosed?
15. What feature of the trophozoite of *E. histolytica* is considered diagnostic of a pathologic condition?
16. What are considered good diagnostic criteria for the cyst of *E. histolytica*?

## 4-2. Flagellates and Ciliates

Humans are hosts to several nonpathogenic, cosmopolitan flagellates species. *Dientamoeba fragilis*, *Chilomastix mesnili*, *Retortamonas intestinalis*, *Enteromonas hominis*, and *Trichomonas hominis*. *Giardia lamblia* is the only intestinal flagellate that is considered pathogenic. A pathogenic trichomonad, *Trichomonas vaginalis*, occurs in the urogenital tract. Ciliates that are hosted by man are less numerous, and the only pathogen known is *Balantidium coli*. Species of the ciliated protozoa are conspicuous because of the hair-like cilia that cover their bodies. They are found in both vertebrate and invertebrate animals. Wave-like movements of the cilia provide the organisms a means of locomotion.

### 622. *Dientamoeba fragilis*

This is a rather unusual organism having the characteristics of both an ameba and a flagellate. In this text, you'll study reasons for this confusion and offer justification as to why it was formerly classified as an ameba but is now classed as a flagellate.

### Life cycle

Originally, *D. fragilis* was classified as an ameba because of its ameba-like locomotion by means in projecting clear pseudopods. However, the organism was unlike other ameba in that it did not have a cyst stage, rapidly rounded up and disintegrated with a drop of temperature, and ruptured when exposed to tap water. It was also unlike other ameba in that 60 to 80 percent of its trophozoites are binucleated and 20 to 40 percent are uninucleated as shown in figure 4-3. Upon close observation with an electron microscope, it was discovered that the organism is actually a flagellate and is now classified as such. Because this organism lacks a cyst stage the conventional wisdom regarding transmission of this organism now believes that the nematodes the pinworm (*E. vermicularis*) and roundworm (*A. lumbricoides*) are the vectors for this organism. Organisms resembling *D. fragilis* have been found in the ova of *E. vermicularis*.

### Causal agent

Despite its name, *Dientamoeba fragilis* is not an ameba but a flagellate. This protozoan parasite produces trophozoites; cysts have not been identified. Infection may be either symptomatic or asymptomatic.

Refer to figure 4-3 for the following references. The complete life cycle of this parasite has not yet been determined, but assumptions were made based on clinical data. To date, the cyst stage has not been identified in *D. fragilis* life cycle, and the trophozoite is the only stage found in stools of infected individuals **1**. *D. fragilis* is probably transmitted by fecal-oral route **2** and transmission via helminth eggs (e.g., *Ascaris*, *Enterobius* spp.) has been postulated **3**. Trophozoites of *D. fragilis* have characteristically one or two nuclei (**1**, **4**), and it is found in children complaining of intestinal (e.g., intermittent diarrhea, abdominal pain) and other symptoms (e.g., nausea, anorexia, fatigue, malaise, poor weight gain).

### Geographic Distribution

*D. fragilis* is found worldwide.

#### DIENTAMOEBIA FRAGILIS INFECTION

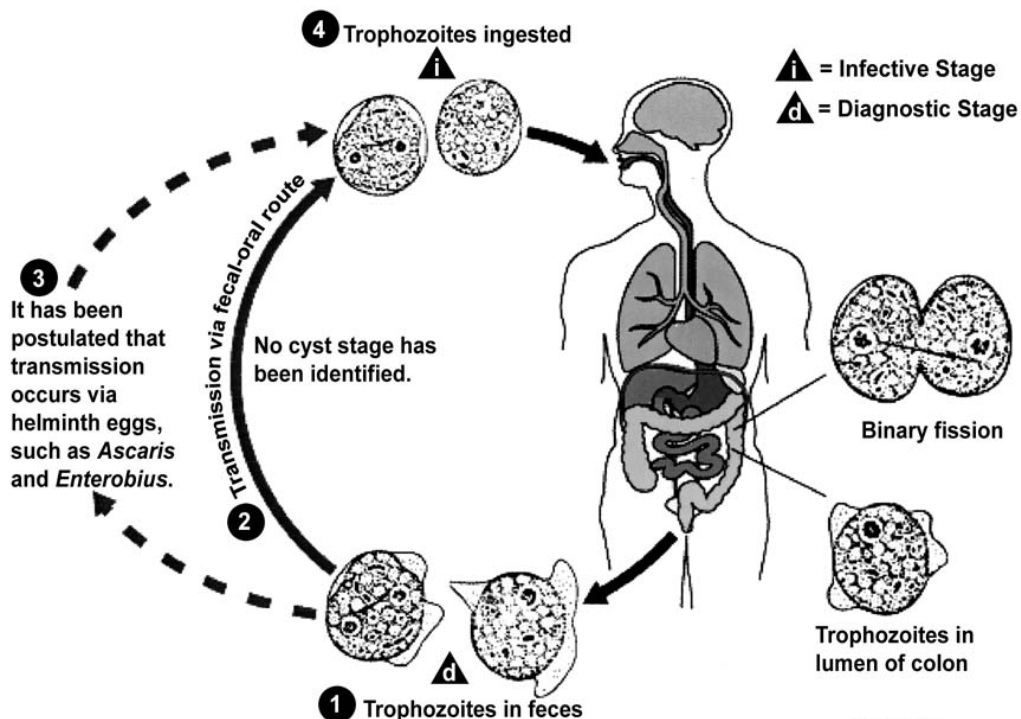


Figure 4-3. The life cycle of *Dientamoeba fragilis*.

### Epidemiology

Since this organism does not form cysts and its trophozoite cannot survive the upper digestive tract, questions naturally arise as to how it is distributed. Its prevalence is reported to range from 1.5 to 20 percent of the population, and under certain institutional conditions (prisons, mental hospitals) where personal hygiene standards are often low and overcrowding is common, the rate of infection may be significantly higher. A correlation between this parasite and another is also seen in abundance in such institutional settings. There is a 10 to 20 times greater than expected association with enterobiasis (pinworm infection), and some parasitologists believe that the organism somehow survives and is transmitted with the ova of *E. vermicularis*. Small, ameboid organisms resembling *D. fragilis* have been found in pinworm eggs, so the evidence to date does point to this nematode acting as a vector for *D. fragilis*. Perhaps future research will prove that a link does exist between the transmission of *D. fragilis* and pinworm. *Ascaris* has been named as a likely suspect for this transmission as well.

### Pathology and clinical manifestations

The organism was formerly thought to be harmless or commensal. New evidence indicates that it may be the causative agent of intestinal discomfort and disease. Symptoms may include diarrhea, abdominal pain, abnormal stools and other indications of intestinal maladies.

### Laboratory diagnosis

Laboratory diagnosis of *D. fragilis* is generally based on finding a large percentage of binucleated, ameba-like trophozoites in permanent, stained smears, as shown in figure 4-3. Each of these trophozoites will vary in diameter from 5 to 15  $\mu\text{m}$ . Their nuclei are distinct in that they lack peripheral chromatin and karyosomes composed of four to eight separate chromatin granules. The uninucleated form of *D. fragilis* is easily confused with trophozoites of *E. nana* or even *I. bütschlii*, unless the karyosome is carefully examined to determine whether it is solid (*E. nana*, *I. bütschlii*) or fragmented (*D. fragilis*).

### 623. *Giardia lamblia*

*G. lamblia* is a pathogenic intestinal protozoan that causes both endemic and epidemic disease—giardiasis. Debate continues regarding the name of this organism, as it is also known as *G. intestinalis* and *G. duodenalis*. Often these three terms are used interchangeably.

### Life cycle

The usual route of infection is by way of unfiltered community water sources, which become contaminated with sewage containing infective mature cysts with four nuclei. The cysts pass through the stomach into the small intestines where, under the influence of the digestive juices, the organism quickly differentiates into two binucleated, 10 to 20  $\mu\text{m}$ , teardrop-shaped trophozoites, each propelled by four pairs of flagella, as shown in figure 4-4. These newly emerged trophozoites attach to the epithelium cells in the crypts of the duodenum and upper jejunum by their ventral sucking disc and begin to multiply. Enormous numbers can build up rapidly in this manner. As is the case with other pathogenic intestinal protozoan organisms, cyst formation by this organism are prompted by the removal of water from the stool. Therefore, depending on how rapidly the stool mass moves through the bowel, trophozoites or cysts will be the predominate form of organism seen in specimens presented to the laboratory.



Figure 4-4. The trophozoite form of *Dientamoeba fragilis*, binucleated on the left and uninucleated on the right.



### Causal agent

*Giardia intestinalis* is a protozoan flagellate (Diplomonadida). This protozoan was initially named *Cercomonas intestinalis* by Lambl in 1859 and renamed *Giardia lamblia* by Stiles in 1915, in honor of Professor A. Giard of Paris and Dr. F. Lambl of Prague. However, many consider the name, *Giardia intestinalis*, to be the correct name for this protozoan. The International Commission on Zoological Nomenclature is reviewing this issue.

Refer to figure 4–5 for the following references. Cysts are resistant forms and are responsible for transmission of giardiasis. Both cysts and trophozoites can be found in the feces (diagnostic stages) ①. The cysts are hardy and can survive several months in cold water. Infection occurs by the ingestion of cysts in contaminated water, food, or by the fecal-oral route (hands or fomites) ②. In the small intestine, excystation releases trophozoites (each cyst produces two trophozoites) ③. Trophozoites multiply by longitudinal binary fission, remaining in the lumen of the proximal small bowel where they can be free or attached to the mucosa by a ventral sucking disk ④. Encystation occurs as the parasites transit toward the colon. The cyst is the stage found most commonly in nondiarrheal feces ⑤. Because the cysts are infectious when passed in the stool or shortly afterward, person-to-person transmission is possible. While animals are infected with *Giardia*, their importance as a reservoir is unclear.

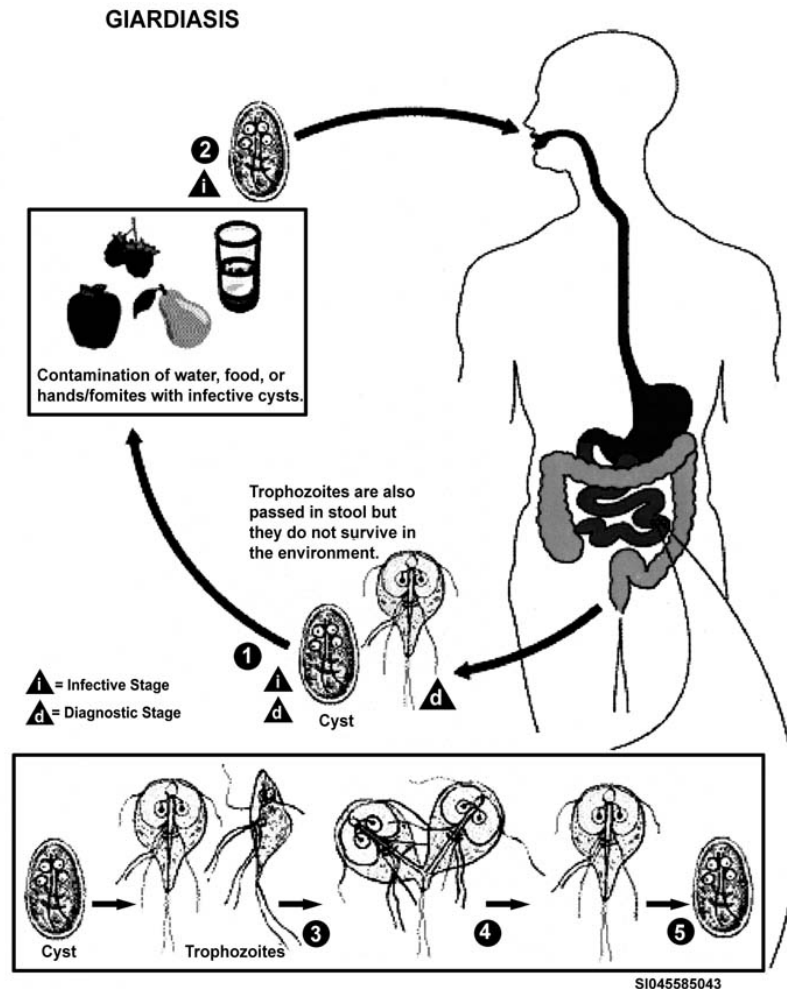


Figure 4–5. Life cycle of *Giardia lamblia*.

**Geographic distribution**

This organism is found worldwide, but is more prevalent in warm climates, and in children.

**Epidemiology**

Giardiasis is highly contagious and the most common pathogenic flagellate of humans. It occurs worldwide, and is more prevalent and severe in children than in adults, especially those children who attend daycare centers. Contaminated water supplies, campers, daycare centers, and male homosexuals link most of the large epidemic outbreaks. In the past, the source of the contamination was human feces, but more recently it has been proven that beavers and wild dogs may act as reservoirs of infection. Since normal levels of chlorination do not kill the cyst of this organism, and cysts are capable of surviving as long as 3 months in fresh water, there is a long-term potential for infection.

**Pathology and clinical manifestations**

A wide range of symptoms characterizes the disease caused by *Giardia*. These include diarrhea, steatorrhea, cramps, malabsorption, and flatulence. High fat content can cause light colored stools. Blood in the stool of a patient with *Giardia* is atypical and rare. Usually this is a self-limiting disease but in some chronic cases there may be multiple relapses.

**Variance in severity**

There is great variance in the severity of the disease associated with this organism. Children generally have more severe infections than adults. It is known that the severity cannot be linked to parasite burden since individuals with light infections may suffer more debilitating symptoms than persons with heavy infections. Apparently, some individuals are more sensitive to the presence of *G. lamblia* than others, and there is evidence that protective immunity can be acquired. Approximately 50 percent of those infected remain asymptomatic; others experience a short-lived acute disease marked by increased mucus production, diarrhea, dehydration, intestinal pain, weight loss, and flatulence. The stool becomes fatty but never contains blood. Still others may develop a chronic infection marked by intermittent acute attacks, and may experience malabsorption of fat-soluble vitamins, folic acid, proteins, and weight loss.

**Laboratory diagnosis**

Diagnosis of *Giardia* is established by recovering the ameboid in stool specimens. The organism is passed in “showers” which is to say that some bowel movements may contain large numbers of the organism and some will contain very little. Multiple specimens over the course of several days need to be collected in order to rule in or rule out the presence of the organism. Direct wet mounts can be used to demonstrate trophozoites and cysts. Permanent stained slides can serve this purpose as well. Concentration techniques are useful in the recovery of cysts. The shape of the *G. lamblia* trophozoite resembles half a pear especially when viewed in its broad dimension, with a tapered posterior end and has two nuclei, giving the appearance of a smiling face with prominent eyes. Upon a side view, the anterior end of the organism is thicker and tapered posteriorly, with the anterior portion consisting of a sucking disk. The flagella are not usually evident in wet mounts or in stained preparations.

**624. *Trichomonas vaginalis***

The trichomonads are small, flagellated, pear-shaped protozoans with prominent undulating membranes. They do not form cysts and, as a result, are usually seen as actively motile trophozoites. These organisms, as a rule, thrive where there is a lack of personal hygiene. The most common human trichomonads are: *Trichomonas vaginalis*, *T. tenax*, and *T. hominis*.

**Life cycle**

*T. vaginalis* is considered a mild pathogen. The other trichomonads are considered nonpathogens. *T. vaginalis*, as shown in figure 4-6 is very typical of its genus in that it exists only as a trophozoite and possesses both an undulating membrane and an axostyle. It differs from other members of its genus in

that its undulating membrane is short, extending less than half the length of its body. Its site of infection in humans is also unique because it is the only flagellate to inhabit the urogenital tract. The life cycle of *T. vaginalis* begins because of contact with vaginal and urethral discharges of infected persons during sexual intercourse and by contact with contaminated articles of clothing or damp wash cloths and clothes. Viable cultures of *T. vaginalis* have been recovered from damp cloth up to 24 hours after inoculation. Infection of newborn infants as they pass through the birth canal is also possible, with females being more susceptible to infection in this manner. Once in the urogenital tract, the trophozoites multiply and form colonies close to the surface of epithelial cells in the vagina and urethra of females, and the urethra, prostate, and seminal vesicles of males.

### Causal agent

*Trichomonas vaginalis*, a flagellate, is the most common pathogenic protozoan of humans in industrialized countries.

Refer to figure 4-6 for the following references. *Trichomonas vaginalis* resides in the female lower genital tract and the male urethra and prostate ❶, where it replicates by binary fission ❷. The parasite does not appear to have a cyst form and does not survive well in the external environment. *Trichomonas vaginalis* is transmitted among humans, its only known host, primarily by sexual intercourse ❸.

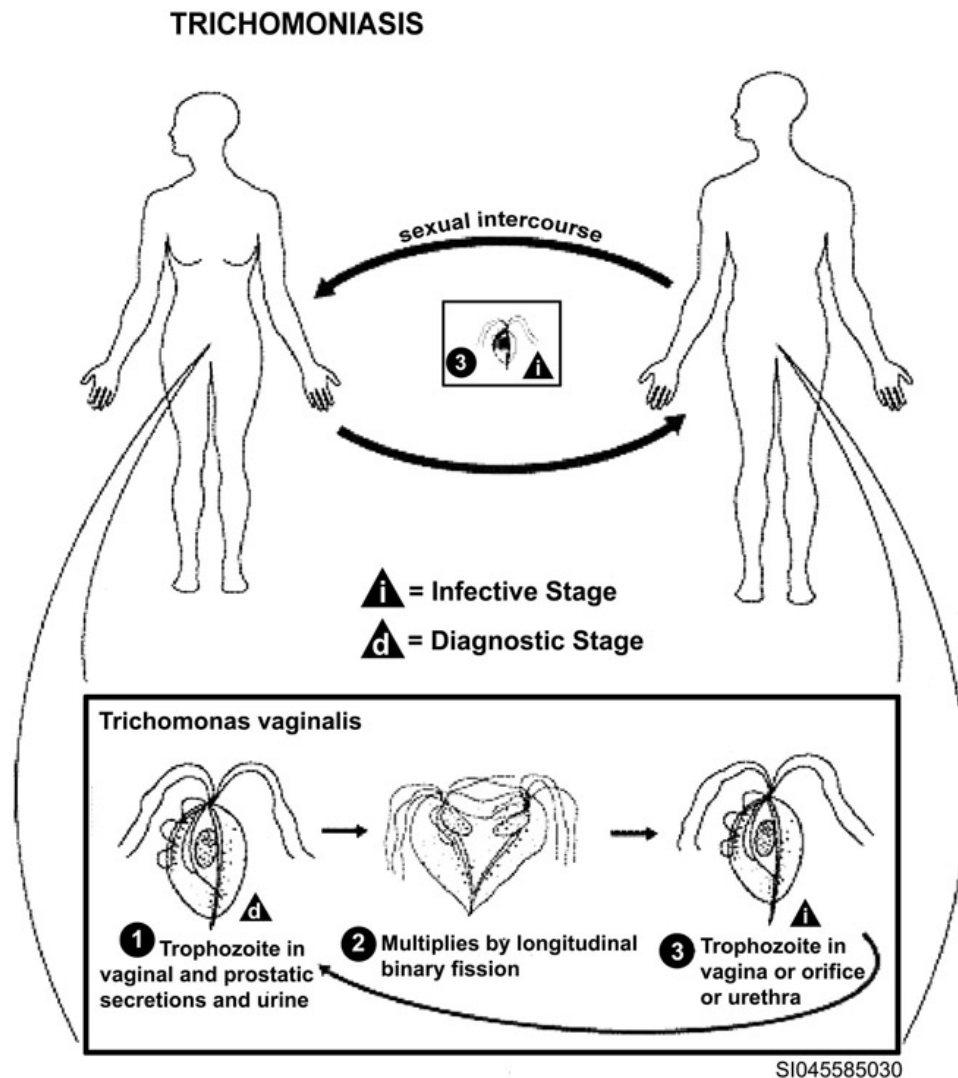


Figure 4-6. Life cycle of *Trichomonas vaginalis*.

**Geographic distribution**

*Trichomonas vaginalis* is found worldwide; however, a higher prevalence exists among persons with multiple sexual partners or other venereal diseases.

**Epidemiology**

*T. vaginalis* has a worldwide distribution and is commonly seen in sexually active people, but most common in females 16 to 35 years of age. In sampled areas of the United States, the prevalence of infection among patients in gynecological clinics has been as high as 50 percent. In various samples of male populations, the prevalence has ranged around five. In the United States there are 1 to 2 million cases reported each year, and worldwide the estimate is 100 to 200 million annually. This is despite the fact that *T. vaginalis* is a somewhat fragile organism that dies when exposed to direct sunlight, drying, or exposed to water for 35 to 40 minutes.

**Pathology and clinical manifestations**

Trichomoniasis is a common, persistent disease that is apt to be asymptomatic in males, although infection may lead to prostatitis, urethritis or inflammation of the seminal vesicles. When such conditions develop, the patient may experience the following:

- A thin, urethral discharge.
- Dysuria.
- Nocturia.
- Pain in the groin.
- An enlarged prostate.

In women, a full range of symptoms may be seen, with a few women being asymptomatic, while most others will present symptoms varying from mild to severe. The disease is commonly characterized by vaginitis, with a profuse yellowish-green, foul-smelling, foamy discharge, and dysuria.

**Laboratory diagnosis**

The laboratory diagnosis of *T. vaginalis* is made by the recovery and identification of the organisms from vaginal or prostatic secretions. Frequently, the organism is found in centrifuged urine specimens. Motile forms can be observed for about 1 hour in urine after the specimen is voided. More than 1 hour after voiding, you will normally only find nonmotile organisms. To recover the parasite from genitourinary secretions, collect the specimens on cotton-tipped swab or platinum loop. Place the swab or loop in a small amount of normal saline and prepare a direct wet mount. Reliable culture techniques are available and should be employed when direct smears are negative. Avoid contamination of urine by feces because *T. hominis* can easily be mistaken for *T. vaginalis*.

**625. *Balantidium coli***

*Balantidium coli* is the largest protozoan parasitizing humans and is the only pathogenic ciliate that inhabits the colon. It is the causative agent of balantidiasis.

**Life cycle**

The usual route of infection with *B. coli*, as shown in figure 4-7, is consumption of water containing infective cysts. Both cysts and trophozoites are passed in the feces of humans, monkeys, and pigs infected with this organism. Once ingested, excystation takes place in the small intestine and colonization, in the large intestine. The trophozoites of *B. coli* are similar to those of *E. histolytica* in that they feed on the starch and bacteria found in the mucosal secretions or invade the mucosa, causing extensive ulceration. *B. coli*, however, never goes beyond the mucosa to cause extraintestinal disease.

**Causal agent**

*Balantidium coli*, a large ciliated protozoan parasite.

Refer to figure 4–7 for the following references. Cysts are the parasite stage responsible for transmission of balantidiasis <sup>1</sup>. The host most often acquires the cyst through ingestion of contaminated food or water <sup>2</sup>. Following ingestion, excystation occurs in the small intestine, and the trophozoites colonize the large intestine <sup>3</sup>. The trophozoites reside in the lumen of the large intestine of humans and animals, where they replicate by binary fission, during which conjugation may occur <sup>4</sup>. Trophozoites undergo encystation to produce infective cysts <sup>5</sup>. Some trophozoites invade the wall of the colon and multiply. Some return to lumen and disintegrate. Mature cysts are passed with feces <sup>1</sup>.

### Geographic distribution

*Balantidium coli* is found worldwide. Because pigs are an animal reservoir, human infections occur more frequently in areas where pigs are raised. Other potential animal reservoirs include rodents and nonhuman primates.

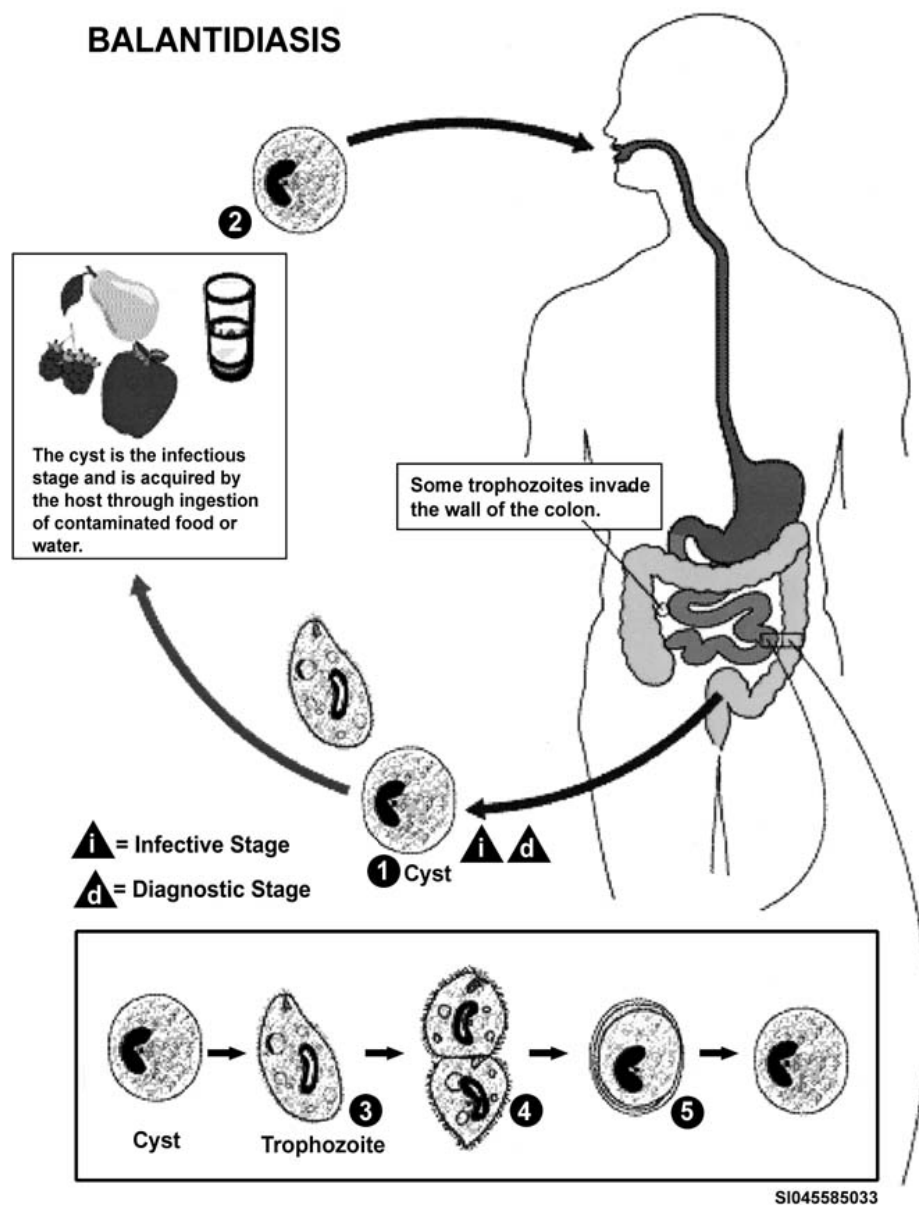


Figure 4–7. Life cycle of *Balantidium*.

**Epidemiology**

*B. coli* is worldwide in its distribution; however, its occurrence is higher in the tropics and areas where there is a close association between humans and pigs. Fortunately, the incidence of infection in man is low, with less than 1 percent of the population infected. As with many other parasites discussed in this unit, infection is linked to lack of proper personal hygiene and environmental sanitation leading to contamination of drinking water.

**Pathology and clinical manifestations**

Balantidiasis is a protozoan infection of the colon characteristically producing diarrhea or dysentery, accompanied by abdominal pain, tenesmus, nausea, and vomiting. Occasionally, the dysentery resembles that of amebiasis, with stools containing much blood and mucus but relatively little pus. However, many infected individuals are asymptomatic, while in a few (especially those already debilitated) the organism may produce the proteolytic enzyme hyaluronidase and invade the mucosal lining, creating a flask-shaped ulcer that favors the development of a secondary bacterial infection. The disease may progress onward, producing necrosis of and sloughing of the tissue overlying mucosal ulcer and, occasionally, perforation of the bowel. Death often occurs at this stage of the disease.

**Laboratory diagnosis**

The trophozoites are large and vary greatly in size, from 40 to 200  $\mu\text{m}$  in greatest dimension, with most measuring 40 to 70  $\mu\text{m}$ . The body is uniformly covered with cilia that become longer at the anterior end near the cytostome. There is an easily seen large macronucleus, and a smaller micronucleus, which is frequently invisible during examination. Numerous food vacuoles will be evident in the cytoplasm. The encysted organism is rounded and, when young, will still show cilia. The cilia disappear as the cyst ages. This organism does not present diagnostic problems other than sometimes being overlooked because of its large size. The identification of this organism's typical cysts or trophozoites in fecal specimens is sufficient for laboratory diagnosis.

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**Self-Test Questions**

After you complete these questions, you may check your answers at the end of the unit.

**622. *Dientamoeba fragilis***

1. What makes *D. fragilis* different from the amoeba?
2. What institutional conditions seem to favor the spread of *D. fragilis*?
3. What nematode may act as a vector for *D. fragilis*?
4. How do you describe the pathogenesis of *D. fragilis*?
5. On what is the laboratory diagnosis of *D. fragilis* usually based?

6. The uninucleated form of *D. fragilis* is easily confused with the trophozoites of what other organisms?

**623. *Giardia lamblia***

1. What is the usual route of infection of *G. lamblia*?
2. What prompts the formation of *G. lamblia* cyst?
3. How common is giardiasis?
4. What is the geographic range of *G. lamblia*, and what age group is most affected?
5. Besides man, what are some other possible reservoirs of infection for *G. lamblia*?
6. What range of symptoms may be displayed by a patient with giardiasis?
7. Can the severity of a patient's symptoms be linked to parasite burden?
8. How is a diagnosis of giardiasis established?
9. Why should a patient suspected of having giardiasis be instructed to collect multiple specimens?

**624. *Trichomonas vaginalis***

1. How is *T. vaginalis* typical of its genus?
2. What is considered unique about *T. vaginalis*?
3. How is a *T. vaginalis* infection acquired?

4. In what sex and age group is *T. vaginalis* most commonly seen?
5. What complications can *T. vaginalis* lead to in males?
6. How do you describe the symptoms of a *T. vaginalis* infection in females?
7. How is the laboratory diagnosis of *T. vaginalis* made?

**625. *Balantidium coli***

1. What is the usual route of infection of *B. coli*?
2. How are the trophozoites of *B. coli* similar to *E. histolytica* trophozoites?
3. Infection with *B. coli* is more common in people in close association with what animal?
4. What is the incidence of human infection with *B. coli*?
5. How do you describe the symptoms of balantidiasis?
6. What enzyme is produced by *B. coli* that enables it to invade the mucosal lining?
7. What consequences may result when *B. coli* invades the mucosal lining?
8. What is the laboratory diagnosis of balantidiasis based upon? The identification of this organism's typical cysts or trophozoites in fecal specimens is sufficient for laboratory diagnosis.
9. How large are the trophozoites *B. coli*?



10. How may the size of *B. coli* trophozoites and cysts present diagnostic problems?

### 4-3. Coccidia and Microsporidia

The intestinal coccidia that parasitize humans belong to the class *Sporozoea* and the subclass Coccidia. They are obligatory tissue parasites that inhabit the mucosa of the small intestine. The four genera are *Isoospora*, *Sarcocystis*, *Cryptosporidium*, and *Cyclospora*. The microsporidian organisms belong to the phylum *Microspora* and are obligate intracellular parasites of invertebrate animals.

#### 626. *Isoospora belli* and *Cryptosporidium* species

Coccidia and microsporidia organisms have interesting and complex life cycles. They reproduce through alternating sexual and asexual cycles. Some of these organisms go through both cycles in one host, while others require two hosts. In the sexual cycle, male and female gametes join to form a zygote that develops into an oocyst. When the oocyst reaches maturity, it ruptures and releases sporozoites. This is the sexual cycle, referred to as *sporogony*. The asexual cycle is initiated by the sporozoites, which in developing, are transformed into trophozoites. Each trophozoite produces a *schizont*. The schizont produces a number of *merozoites*. This is the asexual cycle and is referred to as *schizogony*. Some of the merozoites develop into male gametocytes; others develop into female gametocytes. These unite to start the sexual cycle again.

#### Life cycle of *Isoospora belli*

Human infections with *Isoospora belli*, an intestinal sporozoan parasite, are acquired by ingestion of the product of the parasite's sexual reproduction—the oocyst. Each of the mature, oval oocysts contains two round sporoblasts, which in turn, contain four sausage-shaped sporozoites. The wall of the oocyst is digested in the small intestines releasing the sporozoites. The sporozoites invade the epithelial lining of the small intestine and develop into trophozoites. These trophozoites then begin the process of reproduction through either schizogony (asexual) or sporogony (sexual). The life cycle of *Isoospora* species, including the alteration of sexual and asexual generations and the terminology of the various stages in the life cycle, is similar to the life cycle of the major sporozoan parasites of humans, *Toxoplasma gondii* and *Plasmodium* species. Notable differences are that *Isoospora belli* has only a single host—humans—where all reproduction occurs, and the infection is confined entirely to the intestinal tract. The other organisms differ in that they have true intermediate hosts, in which only the sexual cycle occurs, and the parasites may be found spread throughout the body.

#### Causal agent

The coccidian parasite, *Isoospora belli*, infects the epithelial cells of the small intestine, and is the least common of the three intestinal coccidia that infect humans.

Refer to figure 4-8 for the following references. At time of excretion, the immature oocyst contains usually one sporoblast (more rarely two) ①. In further maturation after excretion, the sporoblast divides in two (the oocyst now contains two sporoblasts); the sporoblasts secrete a cyst wall, thus becoming sporocysts; and the sporocysts divide twice to produce four sporozoites each ②. Infection occurs by ingestion of sporocysts-containing oocysts: the sporocysts excyst in the small intestine and release their sporozoites, which invade the epithelial cells and initiate schizogony ③. Upon rupture of the schizonts, the merozoites are released, invade new epithelial cells, and continue the cycle of asexual multiplication ④. Trophozoites develop into schizonts which contain multiple merozoites. After a minimum of one week, the sexual stage begins with the development of male and female gametocytes ⑤. Fertilization results in the development of oocysts that are excreted in the stool ①. *Isoospora belli* infects both humans and animals.

### Geographic distribution

Worldwide, especially in tropical and subtropical areas. Infection occurs in immunodepressed individuals, and outbreaks have been reported in institutionalized groups in the United States.

### ISOPORIASIS

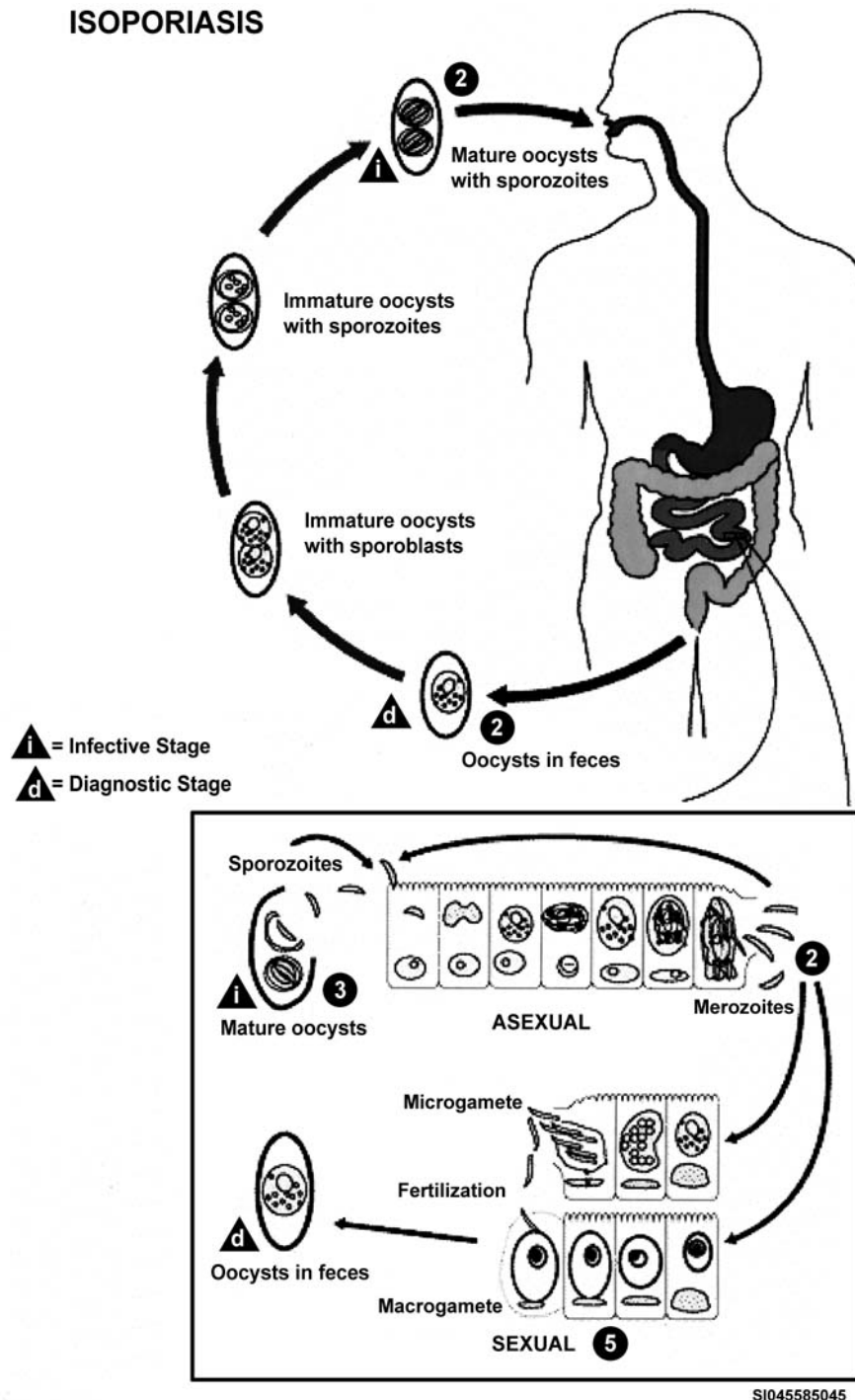


Figure 4-8. Life cycle of *Isospora belli*.

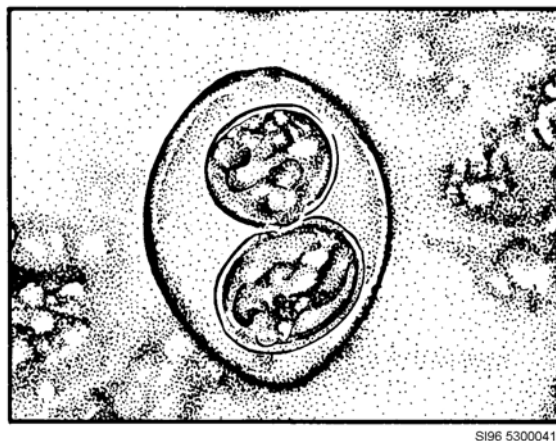
### Epidemiology

Little is known about the exact epidemiology of *Isospora* infection. Presumably, the major features of its mode of transmission are similar to those of other intestinal protozoans, through the ingestion of infective sporozoites from the feces of carnivores or improperly cooked or raw meat.

### Pathology and clinical manifestations

Most infections with *Isospora* are apparently asymptomatic, while clinically manifested cases are generally mild and limited to a few weeks in duration. Cases that are more serious may last for years, and the patient may have severe dysentery accompanied by abdominal pain and weight loss. Many of the clinically manifest cases of *Isospora* have signs and symptoms suggesting a malabsorption syndrome similar to that observed in some cases of giardiasis. Death from overwhelming infections have been reported in immunocompromised patients, especially AIDS patients.

The laboratory diagnosis of *Isospora* infections is based on finding immature oocysts in fecal specimens. The immature or unsporulated oocyst typically measures 20 to 30  $\mu\text{m}$  long by 10 to 19  $\mu\text{m}$  wide, with tapered ends and a smooth, double-layered hyaline wall. The immature oocyst contain only a single, round, cell-like structure, the sporoblast. The immature oocysts complete their development in the external environment, into a mature oocyst. Each oval-shaped mature oocyst contains two spherical cell-like structures—*sporocyst*, as shown in figure 4–9. Because of their relatively large size and distinctive appearance, the oocysts of *Isospora* species are easily observed in iodine-stained wet mounts prepared directly from the fecal specimen or after concentration. Permanent, stained smears are rarely required. The *Sarcocystis* species are similar to the *Isospora* species.



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Figure 4–9. Oocyst of *Isospora*.

### Life cycle of *Cryptosporidium* species

The life cycle of *C. parvum* is similar to *Isospora* species except the organism lacks an intracellular stage and all development occurs on the mucosal cell surface. There seems to be two forms of oocysts in the life cycle of this organism. First, a thin-shelled organism that seems to always rupture while inside the host. Second, a thick-shelled oocyst that typically remains intact and is passed outside the body. The thin-shelled version is thought to be the cause of a majority of autoinfections by this parasite, with the thick-shelled version rarely being the cause of autoinfections.

### Epidemiology

This infection has gained prominence recently due to the susceptibility of immunocompromised patients, especially those with acquired immunodeficiency syndrome (AIDS). These immunocompromised patients suffer greatly from the infection. Immunocompetent patients are usually able to allow the disease to run its course and do recover. When an oocyst is passed in the feces of infected animal, domesticated or livestock, and ingested by a human, the infection is acquired. Most infections are usually sporadic and isolated, however in the 1993, the municipal water system of Milwaukee, Wisconsin was contaminated by the runoff of livestock waste. A large segment of that city's population was infected.

**Pathology and clinical manifestations**

Human infections in individuals who are immunocompetent usually take the form of a self-limited profuse, watery diarrhea, similar to that occurring with *Isospora* species. However, in immunocompromised individuals the diarrhea may be prolonged, causing large fluid losses, anorexia, and sometimes death. The parasite may even disseminate to internal organs, such as the lungs.

**Laboratory diagnosis**

Laboratory diagnosis is based on identifying the oocyst stage from a stool specimen. The organism is readily stained with iodine or acid-fast methods. In the modified Ziehl-Neelsen acid fast procedure the oocysts appear as tiny acid-fast bodies from specimens that are fresh or formalin preserved. Specimens processed in PVA fixative have a tendency to not respond well to acid-fast staining methods. The recommended concentration technique for the recovery of this organism is the Sheather's sugar floatation technique, a method that has been well proven in veterinary parasitology.

**Other tests**

Fluorochrome and carbol fuchsin acid-fast stains are helpful. Also, immunofluorescence and Enzyme-linked ImmunoSorbent Assay (ELISA) diagnostic kits are commercially available.

**Overview of the *Microsporidia***

The microsporidian genera are *Enterocytozoon*, *Septata*, *Nosema*, *Encephalitozoon*, and *Pleistophora*. *Enterocytozoon* and *Septata* are intestinal parasites and the others are found in a variety of tissues. However, *Septata* species may also disseminate to other tissues. These parasites are primarily diagnosed in immunocompromised individuals and microsporidial keratoconjunctivitis has recently been recognized in patients with AIDS. Since the spores are small (1 to 2  $\mu\text{m}$ ) they are rarely found in stool specimens. They are usually identified by Giemsa-stained techniques of intestinal biopsies or electron microscopic studies of biopsied material.

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**Self-Test Questions**

After you complete these questions, you may check your answers at the end of the unit.

**626. *Isospora belli* and *Cryptosporidium* species**

1. How are human infections of *I. belli* acquired?
2. What two types of reproduction occur in the life cycle of *I. belli*?
3. In what stage of development are oocysts of *I. belli* passed from the human body?
4. What are the most notable differences between the life cycle of *I. belli* when compared to the life cycles of other sporozoan parasites?
5. What are the clinical manifestations seen in most cases of *I. belli*?

6. What is the basis for the laboratory diagnosis of an *I. belli*?
7. How does the life cycle of *Cryptosporidium* differ from that of *Isospora* species?
8. Why has interest in human *Cryptosporidium* infections increased?
9. Upon what finding is the diagnosis of cryptosporidiosis usually made?
10. What type of stain is utilized to detect the oocyst of *Cryptosporidium*?
11. What concentration techniques is recommended for the recovery of *Cryptosporidium* oocysts?
12. What are the five microsporidia genera?

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### Answers to Self-Test Questions

621

1. Individual variation in the pH, bacterial flora, and nutritional factors all seem to influence the course of the disease.
2. The study of *E. histolytica* zymodemes.
3. Amebiasis due to *E. histolytica* begins with the ingestion of a mature metacyst with four nuclei.
4. No, if a trophozoite is ingested, it disintegrates in the stomach without producing infection.
5. The metacystic trophozoites can (1) invade the host tissue, (2) live in the lumen of the large intestine without invasion, (3) undergo encystation.
6. As the fecal matter becomes dehydrated, the amoebas are stimulated to encyst.
7. A large glycogen vacuole and chromatoid bars.
8. It enables the cyst to endure unfavorable environmental conditions.
9. The rate at which the fecal mass is expelled from the body.
10. Prevalence closely parallels the level of sanitation.
11. 30 days.
12. Amebic colitis.
13. Amebic abscess of the liver.
14. By radiographic scans, ultrasound, and serological tests.
15. Phagocytosis of erythrocytes.
16. The number and size of nuclei and the appearance of chromatoid bodies are good diagnostic criteria for cyst.

**622**

1. It does not have a cyst stage, rapidly rounded up and disintegrated with a drop of temperature, and ruptured when exposed to tap water.
2. Low personal hygiene standards and overcrowding.
3. *E. vermicularis*.
4. Pathogenesis is not well defined, but diarrhea and mucosal damage may occur.
5. Diagnosis is generally based on finding a large percentage of binucleated, ameba-like trophozoites in permanent, stained smears.
6. *E. nana* and *I. bütschlii*.

**623**

1. Ingestion of unfiltered water from a contaminated source.
2. The removal of water from the stool.
3. It is the most common pathogenic flagellate in man.
4. It occurs worldwide and is more prevalent in children.
5. Beavers and wild dogs.
6. Symptoms can vary from mild diarrhea to full malabsorption syndrome.
7. No.
8. By demonstration of *G. lamblia* trophozoites and/or cysts in fecal specimens.
9. The passage of organisms can vary from day to day.

**624**

1. It exists only as a trophozoite and possesses both an undulating membrane and an axostyle.
2. It is the only flagellate to inhabit the urogenital tract of humans.
3. During sexual intercourse with an infected person and by contact with contaminated articles of clothing or damp clothes.
4. In females, 16 to 35 years of age.
5. Prostatitis, urethritis, or inflammation of the seminal vesicles.
6. Commonly, the disease is characterized by vaginitis, with a profuse yellowish-green, foul-smelling, foamy discharge, and dysuria.
7. By the recovery and identification of the organisms from vaginal or urethral secretions.

**625**

1. Consumption of water containing infective cysts.
2. They may feed on starch and bacteria found in the mucosal secretions, or they may invade the mucosa causing extensive ulceration.
3. Pigs.
4. Less than 1%.
5. Diarrhea or dysentery, accompanied by abdominal pain, tenesmus, nausea, and vomiting.
6. Hyaluronidase.
7. Creation of flask-shaped ulcers that favor the development of a secondary bacterial infection.
8. The identification of this organisms typical cysts or trophozoites in fecal specimens is sufficient for laboratory diagnosis.
9. 40 to 200  $\mu\text{m}$ .
10. They may be overlooked because of their large size.

**626**

1. Infection with *I. belli* is acquired by ingestion of an oocyst.
2. Asexual binary fission (schizogony) and sexual reproduction (sporogony).
3. Oocysts can be passed in the feces in almost any stage of development.

4. The most notable differences are that *Isospora belli* has only a single host, humans, in whom both the sexual and asexual cycles occur; and the infection is confined entirely to one body region, the intestinal tract.
5. Most cases are asymptomatic.
6. Finding the mature or immature oocysts in fecal specimens.
7. *Cryptosporidium* does not have an intracellular stage.
8. Because of the serious consequences of the infection in compromised hosts, especially AIDS patients.
9. Finding the oocyst in fecal specimens.
10. A modified Ziehl-Neelsen acid-fast stain.
11. Sheather's sugar flotation technique.
12. The microsporidian genera are *Enterocytozoon*, *Septata*, *Nosema*, *Encephalitozoon*, and *Pleistophora*.

**Do the unit review exercises before going to the next unit.**

## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter.

49. (621) The **most** common form of extraintestinal amebiasis caused by *E. histolytica* is
  - a. brain abscess.
  - b. liver abscess.
  - c. peritoneal ascites.
  - d. amebomas of the bowel.
50. (621) Serologic tests are positive in about what percent of patients with invasive intestinal amebiasis?
  - a. 50.
  - b. 60.
  - c. 70.
  - d. 80.
51. (622) *Dientamoeba fragilis* has the characteristics of both an ameba and a
  - a. ciliate.
  - b. sporozoa.
  - c. flagellate.
  - d. microspora.
52. (622) There is a correlation between the prevalence of *D. fragilis* and what other parasitic organism?
  - a. *G. lamblia*.
  - b. *T. trichiura*.
  - c. *T. vaginalis*.
  - d. *E. vermicularis*.
53. (623) The **usual** route of infection with *Giardia lamblia* is
  - a. contaminated food.
  - b. unfiltered community water sources.
  - c. sexual contact with an infected person.
  - d. such fomites as damp wash clothes and clothing.
54. (623) What animals act as reservoirs of infection for *G. lamblia*?
  - a. Cats and skunks.
  - b. Muskrats and foxes.
  - c. Raccoons and ducks.
  - d. Wild dogs and beavers.
55. (624) *Trichomonas vaginalis* differs from other members of its genus in that
  - a. it forms a cyst stage.
  - b. it does not have flagella.
  - c. it does not have an axostyle.
  - d. its undulating membrane is short, extending less than half the length of its body.
56. (624) A *T. vaginalis* infection is acquired
  - a. via insect vector or sexual intercourse with an infected person.
  - b. by ingesting contaminated water or through the bite of an insect.
  - c. through contact with contaminated clothing or consumption of contaminated food.
  - d. as a result of sexual intercourse with an infected person or contact with contaminated clothing.



57. (625) What enzyme produced by *B. coli* enables it to invade the mucosal lining of the bowel?
- a. Hydroxylase.
  - b. Hyaluronidase.
  - c. Glutamate dehydrogenase.
  - d. 5-Hydroxyindolacetic acid.
58. (626) The sexual cycle of reproduction of *Isospora belli* is confined entirely to the
- a. liver.
  - b. bloodstream.
  - c. intestinal tract.
  - d. mucus membranes.

## **Student Notes**

## Unit 5. Blood and Tissue Protozoa

|                                                                                                                                                                  |             |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
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| 627. Genus <i>Plasmodia</i> .....                                                                                                                                | 5-1         |
| 628. Diagnosis of <i>Plasmodia</i> .....                                                                                                                         | 5-6         |
| 629. <i>Babesia</i> species .....                                                                                                                                | 5-8         |
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| 634. <i>Leishmania tropica</i> complex, <i>Leishmania mexicana</i> complex, <i>Leishmania braziliensis</i> complex, and <i>Leishmania donovani</i> complex ..... | 5-26        |

**I**N THIS unit you will study both sporozoan and flagellate organisms that infect the blood and tissue of man. Most of these organisms, with the exception of *Pneumocystis jiroveci* (formerly *carinii*) and *Toxoplasma gondii*, are not commonly found in the United States. However, the possibility that these organisms could gain a foothold and establish a new endemic area does exist. The following two factors favor this occurrence: suitable vectors for some of these organisms already exist, and the importation of suitable vectors for other types is a possibility, and tourists visiting endemic areas have been found to harbor parasitic infections upon returning to the United States. In addition, many of these parasites can have a devastating effect on military personnel and operations.

### 5-1. Sporozoa

Sporozoans lack cilia or flagella and typically have complex life cycles requiring more than one host. Malaria is likely the best-known organism in the phylum. Sporozoa can occur as an acute or chronic protozoan infection characterized by a life cycle that includes both sexual and asexual reproduction. Geographically, it generally occurs between 45° north and 40° south latitude (tropics and subtropics). The majority of human malarial infections are caused by the four species of the genus that are addressed individually in this section.

#### 627. Genus *Plasmodia*

Malaria, a very debilitating disease, has plagued man throughout recorded history and has probably had a greater impact on world history than any other infectious disease. It was prevalent during the Greek and Roman empires. The disease was responsible for over 25 percent of all hospital admissions during the Civil and Spanish-American wars. Epidemics of malaria severely hampered allied operations in the Pacific and Asian theaters of World War II. American troops stationed in Korea during the police action of the early 1950s were exposed to its ravages, and for U.S. Forces in Southeast Asia, it was one more enemy they had to fight.

#### Life cycle

Many different kinds of birds and mammals can contract malaria. However, specific species of plasmodia infect specific animals. The species are similar in many respects, but each possesses qualities or characteristics that differentiate it from the others. Before you study the individual characteristics of each of these species, we will familiarize you with the way these organisms reproduce and how humans transfer them. The reproductive cycle of the malarial parasite is similar to that of other members of the class *Sporozoea*. It consists of alternating sexual and asexual cycles. In contrast to the sexual and asexual cycles of *Isospora* that take place in a single host, the sexual and asexual cycles of the plasmodia take place in two separate hosts—humans and mosquitoes. The asexual portion of the cycle that takes place in humans is illustrated schematically in figure 5-1.

### Intra-human asexual cycle of reproduction

The disease starts in humans because of the bite of an infected female *Anopheles* mosquito. The mosquito needs a blood meal for nourishment prior to laying eggs. After the mosquito inserts its proboscis into a capillary, it pumps saliva into the capillary to prevent coagulation of the blood. Sporozoites from the salivary glands deposit into the person's circulation. Once sporozoites get into the circulation, they make their way to the liver, where they invade parenchyma cells. It is estimated that, within 30 minutes to 1 hour after a bite, the sporozoites have completely disappeared from the blood.

### Exoerythrocytic stage

In the liver, the parasite begins the *exoerythrocytic stage*. Here the sporozoites mature into *schizonts*. The schizonts develop daughter merozoites. At one time, it was thought that some of these merozoites reentered other parenchymal cells in the liver and continued the exoerythrocytic phase. To the contrary, however, current evidence suggests that daughter merozoites cannot re-invade the liver cells and repeat the process. Still, in infections of *P. vivax* and *P. ovale*, some of the invading sporozoites give rise to exoerythrocytic forms (hypnozoites) that remain dormant in the liver for up to 5 years before beginning exoerythrocytic schizogony. This delayed production of merozoites from hypnozoites is presumably responsible for recurrence of active *P. vivax* or *P. ovale* malaria long after a patient has recovered from the initial clinical attacks of the disease.

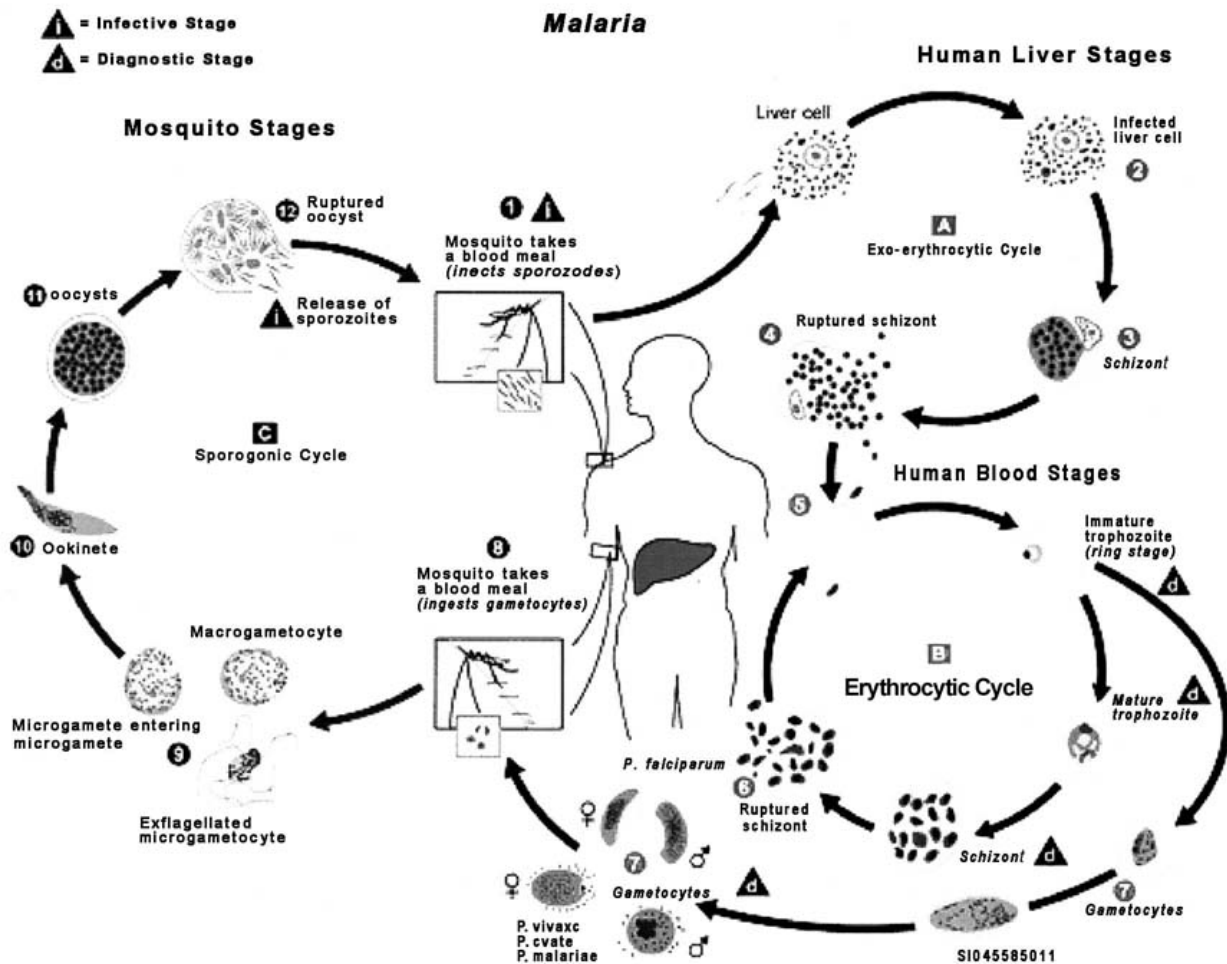


Figure 5-1. Life cycle of plasmodia.

## Causal Agents

Blood parasites of the genus *Plasmodium* are numerous. There are approximately 156 named species of *Plasmodium* which infect various species of vertebrates. Four are known to infect humans: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*.

Refer to fig. 5-1 for the following references. The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host ❶. Sporozoites infect liver cells ❷ and mature into schizonts ❸, which rupture and release merozoites ❹. (Of note, in *P. vivax* and *P. ovale* a dormant stage (hypnozoites) can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony A), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony B). Merozoites infect red blood cells ❺. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites ❻. Some parasites differentiate into sexual erythrocytic stages (gametocytes) ❼. Blood stage parasites are responsible for the clinical manifestations of the disease.

The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal ❽. The parasites' multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes ❾. The zygotes in turn become motile and elongated (ookinetes) ❿ which invade the midgut wall of the mosquito where they develop into oocysts ⓫. The oocysts grow, rupture, and release sporozoites ⓬, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle ❶.

### Erythrocytic stage

The series of events that occur within red blood cells is referred to as the *erythrocytic stage*. After entering the red blood cell, the merozoite develops into an ameba-like trophozoite. This trophozoite ingests the hemoglobin of the cell. The protein portion of hemoglobin uses the parasite as nourishment; the iron portion is not used. This iron collects in the parasite and appears as dark granules referred to as *malarial pigment*. The trophozoite continues to develop until a schizont is produced. This schizont develops numerous merozoites, which are released into the circulation when the red blood cell ruptures. Some of these merozoites enter other red blood cells and start the erythrocytic stage again. The other merozoites enter red blood cells and develop into male or female gametocytes. The parasites suck up in the blood meal of a mosquito. Within the mosquito, the parasite reproduces through the sexual cycle.

### Intra-mosquito sexual cycle of reproduction

Gametocytes sucked up in the blood meal are either male microgametocytes or female macrogametocytes. They are transported to the mosquito's midgut. Each microgametocyte eventually produces several flagellated microgametes (male sex cells). Each macrogametocyte transforms into a single macrogamete (oocyte), which is an unfertilized female sex cell. The union of microgamete and oocyte results in a free-swimming *ookinete*, which migrates into the stomach wall and becomes an encysted oocyst. Within itself, the oocyst produces numerous sporozoites. When ripe, the oocyst ruptures and releases the sporozoites, and they migrate to the salivary glands. They escape from there when the mosquito feeds. Study of the reproductive cycle of malarial parasites in man and the mosquito has contributed greatly to efforts to control the disease. The peculiar rhythmic fevers seen in malaria coincide with the rupture of red blood cells and the release of merozoites from mature schizonts. The period between fever peaks varies according to which species causes the infection.

The table gives the time intervals of the fever spikes for each of the species, along with a comparison of several other features that will aid you in differentiating them.

| Characteristic                                | <i>P. vivax</i>                             | <i>P. malariae</i>                                                                 | <i>P. falciparum</i>                                           | <i>P. ovale</i>                                                  |
|-----------------------------------------------|---------------------------------------------|------------------------------------------------------------------------------------|----------------------------------------------------------------|------------------------------------------------------------------|
| Duration of Cycle                             | 44–48 hours                                 | 72 hours                                                                           | 36–48 hours                                                    | 48 hours                                                         |
| Relapses                                      | Yes                                         | No, but long-term recrudescences occur                                             | No long-term relapses                                          | Possible, but usually spontaneous recovery                       |
| Schüffner's dots                              | Usually present, except in early ring forms | None                                                                               | None, occasionally Maurer's dots present                       | Yes, dots may be larger and darker than those of <i>P. vivax</i> |
| Developmental stages seen in peripheral blood | All                                         | All, but ring stage is brief, mostly growing and mature trophozoites and schizonts | Young ring forms and no older stages, occasionally gametocytes | All                                                              |
| Color of cytoplasm                            | Decolorized, pale                           | Normal                                                                             | Normal, bluish tinge at times                                  | Decolorized, pale                                                |
| Appearance of growing trophozoites            | Multi-shaped irregular amoeboid parasite    | Compact rounded or band-shaped solid forms                                         | Heavy ring forms                                               | Ring shape                                                       |
| Number of merozoites in mature schizont       | 12–24, usually 16                           | 6–12, usually 8                                                                    | Not seen in peripheral blood                                   | 3/4 of cell occupied by 8–12, usually 8                          |
| Malarial pigment in Gametocyte                | golden to light brown                       | darker and coarser                                                                 | heavy black pigment                                            | golden to light brown                                            |

### Epidemiology

Malaria is a widespread, high-impact disease of epidemic proportions in many tropical and subtropical parts of the world. An estimated 250 million people are afflicted throughout the world. At least 1 million people, most of them children, die each year from malaria. Fortunately, several factors limit the prevalence of malaria.

### Suitable hosts

The number of suitable hosts consists of the available nonimmune hosts. This involves the development of immunity and resistance from immunity.

### Development of immunity

The development of some protective acquired immunity is evident in individuals who have had malaria.

### Resistance from immunity

Genetic resistance distinct from acquired immunity is also a factor that limits susceptibility to certain species of malaria. The most famous example of this phenomenon is that of sickle cell anemia. The presence of abnormal hemoglobin S. inhibits the growth and development of *P. falciparum* in the erythrocyte. Another example of genetic resistance to *P. falciparum* is favism. Since plasmodia are entirely dependent on the G6PD of the host cell, and persons with favism are genetically deficient in G6PD, these individuals show greater resistance to infection.

### Suitable vector

The presence of a suitable vector takes the form of any of 390 species of anopheline mosquitoes. Their abundance, feeding preferences, and patterns of activity all influence the malaria in a given area. Much depends on the many preferences of the indigenous mosquitoes such as breeding locations, animal or human blood, and territory or range of the insect.

**Weather conditions**

Local weather conditions; that is, temperature fluctuations and well-defined wet and dry seasons will be reflected in the number of seasonal malarial infections, with the incidence of disease increasing in the warm wet months. *P. vivax* and *P. malariae* are more common in temperate climates and found year round. *P. falciparum* and *P. ovale* are mostly limited to tropical and subtropical climates and are greatly affected by prevailing weather conditions. *P. falciparum* and *P. ovale* will usually be more prevalent in the wet season. The incidence of *P. falciparum* and *P. ovale* is limited also by the fact they do not have a dormant liver stage, which helps ensure survival in areas where mosquito breeding occurs only a few times annually.

**Pathology and clinical manifestations**

The major pathologic and clinical manifestations of malaria are associated with the erythrocytic phase of the parasites' life cycle. These manifestations are:

- Headache.
- Anorexia.
- Muscle ache.
- Nausea.
- Chills, and fever—the so-called “hot” stage of the disease and the anemia that accompanies this parasitic infection.

The host's inflammatory response to the parasite is responsible for the chills and fever, and the enormous destruction of red blood cells results in the anemia. A number of mechanisms cause anemia. The anemia can be caused by a number of mechanisms, such as:

1. Direct red blood cell lysis due to the life cycle of the parasite.
2. Splenic removal, both infected and uninfected red blood cells coated with immune complexes.
3. Autoimmune lysis of coated infected and uninfected red blood cells.
4. Decreased incorporation of iron into heme.
5. Increased fragility of red blood cells.
6. Decreased red blood cell production from bone marrow suppression.

**Severity**

The severity of a particular malarial infection relates to the species of malaria causing the infection. *P. falciparum* is the most serious form, with *P. vivax* and *P. ovale* being the least dangerous. The severity of disease links to the particular age of the erythrocyte preferred by the four species of malaria infecting man. *P. vivax* and *P. ovale* prefer to infect younger erythrocytes (reticulocytes), while *P. malariae* prefers older cells. The fact that *P. falciparum* has no age preference is partially responsible for its virulence, marked by thrombosis of the capillaries, ischemia, and tissue anoxia within affected organs.

**Abnormal pigmentation**

Abnormal pigmentation, external and internal, is another clinical manifestation of malaria. External pigmentation, in the form of jaundice, is sometimes observed as the body's capacity to excrete the excess bilirubin created by the hemolytic process is exceeded. Hemozoin, or malarial pigment, an insoluble, brown-black end product of the malarial parasites metabolism of hemoglobin, also accumulates. It is taken up in the reticuloendothelial system (mononuclear phagocytic system). The accumulation of hemozoin will cause the liver, spleen, and brain to become black in color. An organ will display discoloration only when large numbers of *P. falciparum* infected red cells become sequestered in its capillaries. Some of the organs that are most prone to this condition include the

brain, liver, adrenal glands, lungs, gastrointestinal tract, and the kidneys. Repeated infections may result in a generalized intravascular hemolysis commonly known as black water fever.

### **Summary**

In summary, malaria can produce allergic manifestations, anemia, congestion of capillaries, enlargement of the spleen, and congestion of the kidneys, stomach, and intestines. It also produces enlargement, congestion, and pigmentation of the liver and other internal organs and a decrease in the functioning of the bone marrow, and fatty degeneration of the heart.

### **628. Diagnosis of *Plasmodia***

The laboratory diagnosis of malaria depends to some extent on the physical manifestations of the disease; however, the most important diagnostic feature is demonstration of the parasite in stained smears of peripheral blood.

#### **Blood collection**

Demonstrating the parasite is best accomplished by collecting morning and evening blood samples for three consecutive days. It is also best to use blood that has not had anticoagulants added to it. Thick and thin blood films collected directly from a free-flowing finger puncture are ideal. Once acquired and properly stained, these smears can be used to confirm a diagnosis of malaria. The thick films, although more difficult for most technicians to read due to the compression of the parasite, which distorts its characteristic appearance, and the presence of debris and artifacts, is the best way to screen multiple samples. If you face an outbreak of malaria, it will quickly become apparent that efficient screening of thick films is a necessary skill to acquire because six specimens per patient will provide you with an impressive addition to your normal workload. Once you confirm the presence of a malarial infection from the thick film, you can then use the thin film to determine the type or types of malarial organisms present. Simultaneous infection with more than one species of malaria is occasionally seen. Accordingly, you will have to be familiar with the key morphological characteristics unique to each species. An accurate and timely laboratory diagnosis of the species of malaria causing infection does impact the treatment regime adopted by the attending physician. It will also reduce the possibility of the development of serious complications resulting from a missed or delayed diagnosis. It is especially important to provide the health care provider both accurate and timely laboratory data where *P. falciparum* infections exist, since serious, even life-threatening, complications can develop rapidly in such a patient.

#### **Microscopic morphology**

Now let's review those technical details that should enable you to speciate malarial parasites based on their microscopic morphology. We especially emphasize the schizont stage, since, with the exception of *P. falciparum*, this is the most reliable stage for special study because each species has a unique number and arrangement of merozoites in this stage of its development.

#### ***Plasmodium vivax***

*P. vivax* is the most widely distributed of the *Plasmodium* parasites and found in all tropical zones of the world. The disease caused by an infection of *P. vivax* is called "tertian" malaria, a term that refers to the 48-hour period required for schizotonic development in the blood to take place. Fever spikes occur in 48-hour intervals (or every third day). Morphologically, the stages of development can be summarized as follows:

1. The young trophozoite (ring form) usually has a large chromatin dot. The cytoplasmic circle is large and pale blue in color.
2. A growing trophozoite has an irregular outline, with several finger-like pseudopodia. Fine, yellow-brown granules (malaria pigment) may be present in the cytoplasm, and the chromatin dots are single and compact.



3. In the large trophozoite, the parasite practically fills the enlarged red blood cell. Chromatin is seen as an abundant, loose or compact mass.
4. Chromatin, in an immature schizont, is divided into two or more irregular masses. Pigment is generally clumped into several large particles.
5. A mature schizont (segmented) is divided into 12 to 24 merozoites, with oval nuclei surrounded by a similarly shaped mass of cytoplasm.
6. The macrogametocyte is a circular or ovoid mass, with homogeneous cytoplasm that has no vacuoles. The chromatin mass is single, small, compact, dark red in color, and eccentric in position.
7. Usually, the microgametocyte is circular when fully mature. Cytoplasm stains light blue, and the chromatin mass is large, diffuse, light red in color, and located at or near the center of the organism.

Specific features of *P. vivax* can be seen in drawings in foldout 1, found at the end of this volume. The drawings show the appearance of the various stages as seen in the red blood cells on stained preparations.

### ***Plasmodium falciparum***

*P. falciparum* produces a disease called “malignant tertian” or “estival-autumnal” (summer-autumn) malaria. Here the ring forms are smaller than those of the other species described in this unit. Foldout 2, also found at the end of this volume, illustrates the appearance of *P. falciparum*. It is restricted to tropical and subtropical climates since it does not have a dormant liver stage that allows it to survive in more temperate areas that have seasonal mosquito breeding patterns. Following are some features of *P. falciparum*:

1. Ring forms with single or double-chromatin dots, which are often seen in peripheral smears. Multiple rings may be seen. This form and the gametocyte are the only stages normally found in the peripheral blood. Of the two, the banana or sausage-shaped gametocyte is the best criterion for speciation. These gametocytes are commonly found in the peripheral blood and may appear to be extracellular even in thin blood smears.
2. Trophozoites, larger than ring forms, are rarely observed in peripheral blood. The cytoplasm appears compact and light blue in color. Very dark pigment is scattered throughout the cytoplasm.
3. Pre-segmenting schizonts are rarely observed in peripheral blood. If schizonts are found, the parasite is small and its pigment is usually clumped in one small, dark mass.
4. A mature schizont, or segmented, is rarely seen in peripheral blood. It is divided into 8 to 24 merozoites and fills about two-thirds of a normal-sized blood cell.
5. Macrogametocytes are long, slender, and sausage-shaped, with a concentrated mass of dark pigment near the center surrounding a dark red chromatin mass. These are commonly observed in peripheral smears.
6. The cytoplasm of the microgametocyte is generally paler in color than that in the macrogametocyte. The parasite is broader, shorter, and has more rounded ends than the macrogametocyte does. Heavy granules of pigment are present. These forms are commonly observed in peripheral smears.

### ***Plasmodium malariae***

*P. malariae* require 72 hours to complete the cycle in man. The term “quartan malaria” is often used to denote an infection with this species of organism. It is not as prevalent, nor is it as widely distributed as *P. vivax* and *P. falciparum*. The parasite is illustrated in foldout 3, likewise found at the end of this volume. *P. malariae* presents the following appearance in stained smears:

1. The ring form contains a single, heavy chromatin dot. The cytoplasmic circle is small and compact.
2. Cytoplasm in the growing trophozoite is compact and frequently in the form of a trapezoidal-shaped band across the center of the red cell. Coarse, dark-brown or black pigment granules are often present.
3. The large trophozoite fills, or almost fills, a normal-size red blood cell. Pigment granules are large, dark, and generally arranged peripherally. The cytoplasm is dense, dark blue, and often in the form of a broad band.
4. In the pre-segmenting schizont, the chromatin divides into a number of masses. The cytoplasm is dense and dark blue, and the pigment is dark and evenly distributed throughout the parasite.
5. A mature schizont, or segmented, has 6 to 12 merozoites, usually in a single circle surrounding a large clump of hemozoin (decomposed hemoglobin) granules. This is called the *rosette* or *daisy* form.
6. The macrogametocyte is circular or ovoid and regular. The cytoplasm is dense, dark blue, and contains abundant, coarse, dark pigment. It has chromatin that is similar to that in *P. vivax*.

### ***Plasmodium ovale***

The incidence of *P. ovale* is very low. The parasite is almost completely limited to parts of West Africa. All of the stages found in *P. vivax* and *P. malariae* are also found in *P. ovale*. In certain respects, an infection of *P. ovale* resembles *P. vivax*; whereas in other respects it is more like *P. malariae*. The erythrocytic cycle requires 48 hours. Schüffner's stippling is observed more markedly in cells infected with *P. ovale* than in those infected with *P. vivax*. As the trophozoite matures, the cytoplasm becomes relatively condensed, as it is in *P. malariae*. The red blood cells that are infected become oval-shaped. They may be of normal size or slightly enlarged, but they are not usually paler than normal. There is not much pigment, and when present, it is light brown. The mature schizont contains 6 to 12 merozoites that surround a central mass of pigment. The gametocytes are very much like those of *P. malariae*, but they can be differentiated in thin blood films by the presence of Schüffner's granules (dots) in the cells. Differentiation from the same stage of *P. vivax* is made on the basis of the denser and more compact makeup of *P. ovale*.

### **629. *Babesia* species**

*Babesia* is also a blood parasite that causes human infection. It was first discovered in 1888 in cattle and in 1893 was recognized as the cause of Texas cattle fever transmitted by ticks. There are three species:

1. *B. microti*.
2. *B. equi*.
3. *B. bigemina*.

### **Life cycle**

*Babesia* species are widely distributed in mammals and the intermediate hosts are ticks. Refer to figure 5-2 for a diagram of the life cycle of this organism, which is similar to *Plasmodium*. The only exceptions are that there are no exoerythrocytic or sexual stages in *Babesia* as exists in *Plasmodium*. The most common form of the *Babesia* parasite found in humans reveal numerous intraerythrocytic trophozoites, nearly identical to the tiny rings of *P. falciparum*. An asexual reproductive form with two or four daughter cells linked together within infected erythrocytes (forming a characteristic "Maltese cross"), may also be found, but these are far less common than in the trophozoite stage.

# Babesiosis

[*Babesia microti*] [*Babesia divergens*]

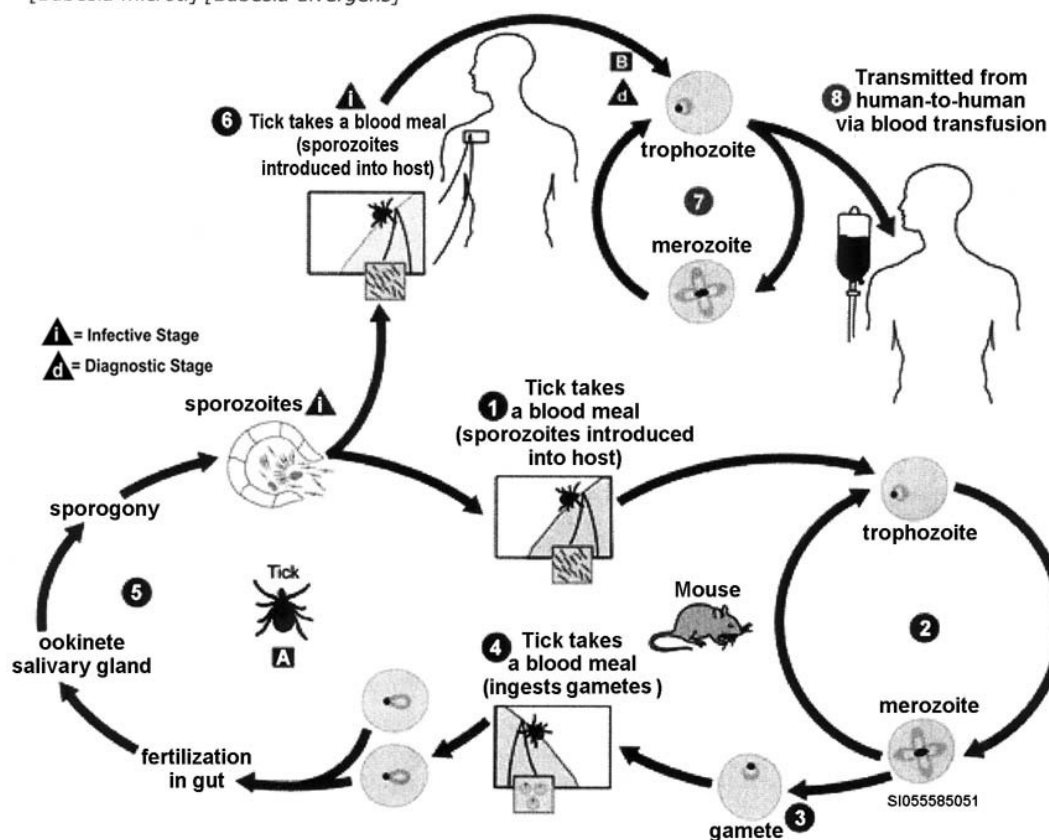


Figure 5-2. Life cycle of Babesiosis.

## Epidemiology

The geographic distribution for *B. microti* is North America and Europe; for *B. equi* it is Europe, Russia, Africa, Asia, India, North and South America; and for *B. bigemina* it is the tropics and subtropics throughout the world. Babesia is no longer rare in the U.S., with most cases caused by *B. microti*. Most of these cases were found on coastal islands in New England. In addition to the northeastern portion of the country, *B. microti* is also found in the western U.S. as well.

## Pathology and clinical manifestations

Infections have been acquired by tick bite and by blood transfusion. The severity of the infection is more marked in individuals who are immunosuppressed or who lack a spleen, and in the elderly. Since the tick species of *Ixodes dammini* transmits both babesiosis and Lyme disease (caused by *Borrelia burgdorferi*), patients with either disease should be carefully evaluated for the presence of the other in areas where both diseases and the vector overlap. Symptoms may resemble that of malaria so differentiation of the two diseases becomes critical.

## Laboratory diagnosis

There are three methods available for the diagnosis of babesiosis:

1. Thick and thin Giemsa stained blood films.
2. Hamster inoculation.
3. Serological testing.

Always examine thick blood smears since the parasite burden may be low when the patient first exhibits symptoms of infection. The thick film will be reminiscent of a malarial film since *B. microti* usually has a very small nucleus and thin cytoplasm much like *P. falciparum*. In the thin smear, the parasitic forms resemble small malarial rings. As the organism grows, it becomes easier to differentiate from malaria since there is no pigment or erythrocyte enlargement, no synchrony, and no schizonts nor gametocytes seen.

### **630. *Toxoplasma gondii***

Toxoplasmosis is a disease with a rather obscure beginning; nevertheless, it has risen to a prominent place in the field of parasitology. The discovery of the organism preceded the discovery of its clinical significance. The organism was originally isolated in 1908 from an obscure desert rodent native to Tunis, called the *gondii*, from which it takes its name. Once its clinical significance was realized, researchers found serological evidence of its worldwide distribution and range of prevalence. Initially, the greatest concerns surrounding this disease were the serious and sometimes fatal effects of this organism due to congenital transmission across the placental barrier. More recently, its impact on immunosuppressed or immunocompromised patients, such as those with transplants or AIDS patients, has become the center of concern.

#### **Life cycle**

*T. gondii*, as shown in figure 5-3, is an obligate intracellular parasite with a complex life cycle. It is closely related to other species in the subclass *Coccidia* and has certain similarities to malarial parasites in this subclass. In the definitive or final host, which is the domestic cat or another member of the family *Felidae*, a two-stage life cycle takes place.

#### **Causal Agent**

*Toxoplasma gondii* is a protozoan parasite that infects most species of warm-blooded animals, including humans, causing the disease toxoplasmosis.

Refer to figure 5-3 for the following references. Members of the cat family (*Felidae*) are the only known definitive hosts for the sexual stages of *T. gondii* and thus are the main reservoirs of infection. Cats become infected with *T. gondii* by carnivorousism ❶. After tissue cysts or oocysts are ingested by the cat, viable organisms are released and invade epithelial cells of the small intestine where they undergo an asexual, followed by a sexual cycle, and then form oocysts which are excreted. The unsporulated oocyst takes 1 to 5 days after excretion to sporulate (become infective). Although cats shed oocysts for only 1 to 2 weeks, large numbers may be shed. Oocysts can survive in the environment for several months and are remarkably resistant to disinfectants, freezing, and drying, but are killed by heating to 70°C for 10 minutes.

Human infection may be acquired in several ways: A) ingestion of undercooked infected meat containing *Toxoplasma* cysts ❷; B) ingestion of the oocyst from fecally contaminated hands or food ❸; C) organ transplantation or blood transfusion; D) transplacental transmission; E) accidental inoculation of tachyzoites. The parasites form tissue cysts, most commonly in skeletal muscle, myocardium, and brain; these cysts may remain throughout the life of the host.

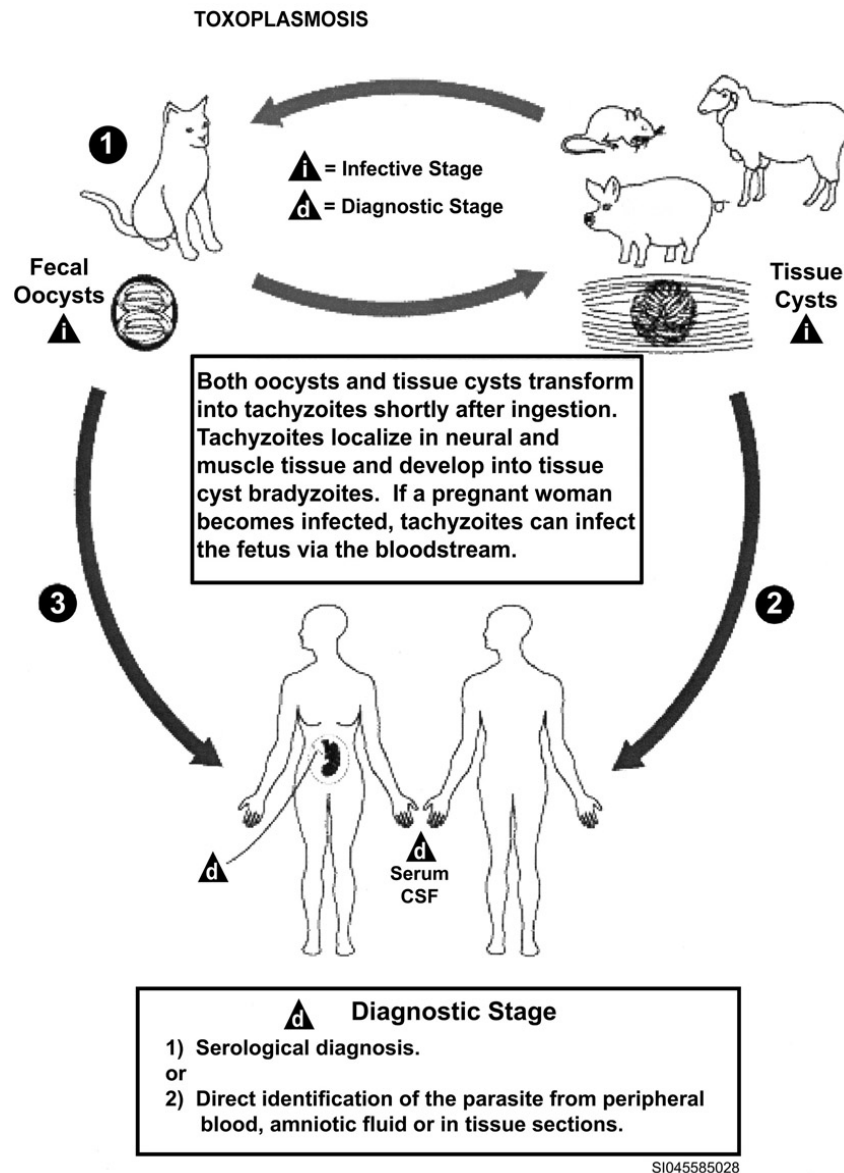


Figure 5-3. Life cycle of *Toxoplasma gondii*.

### Geographic Distribution

Serologic prevalence data indicate that toxoplasmosis is one of the most common of human infections throughout the world. Infection is more common in warm climates and at lower altitudes than in cold climates and mountainous regions. High prevalence of infection in France relates to a preference for eating raw or undercooked meat, while high prevalence in Central America relates to the frequency of stray cats in a climate favoring survival of oocysts. The overall seroprevalence in the United States as determined with specimens collected by the third National Health and Nutritional Assessment Survey (NHANES III) between 1988 and 1994 was found to be 22.5 percent, with seroprevalence among women of childbearing age (15 to 44 years) of 15 percent.

### First stage

The first stage is referred to as the *enteric* stage. In this stage, both an asexual (schizogony) and a sexual cycle (sporogony) occur in the epithelial cells of the small intestines of the cat. The sexual cycle occurs only in cats. The asexual cycle contains the following steps of development:

1. The ingestion of oocysts from the feces of another cat or from the tissues of a reservoir host, such as rodents or birds.
2. The rupture of the oocysts and freeing of its sporozoites, which then infect epithelial cells of the intestine (where they are transformed into trophozoites).
3. The asexual division of the trophozoites (schizogony) and production of schizonts composed of many individual daughter cells called *merozoites*.
4. The rupture of the cell and release of the merozoites.

At this point, stage one of the life cycle, two things may occur:

1. The merozoites may invade epithelial cells and repeat the cycle, or
2. Some of the trophozoites may initiate a sexual reproductive cycle by developing into either male or female sex cell microgametocytes or macrogametocytes, which combine to form immature oocysts.

The oocysts, which are passed in the cat's feces, require 1 to 5 days to mature in the external environment, where they may remain viable for 18 months.

### ***Second stage***

The second stage of the life cycle of *T. gondii* is characterized by multiplication of trophozoites in cells outside the intestinal tract. The infection is acquired in the same manner as in the first stage life cycle, by the ingestion of either pseudocysts or oocysts. The difference between stages one and two is that, in the second, the infective stage released from these cysts penetrates the intestinal wall and begins to invade cells throughout the body, especially white blood cells.

### **Epidemiology**

*T. gondii* infects many different birds and mammals and is most prevalent in warm, moist climates, but all inhabited continents of the world, from time to time, report this organism. As mentioned earlier, serological evidence indicates that the prevalence varies greatly, ranging from 1 to 70 percent worldwide, and 20 to 70 percent in different areas of the United States. Many of the individuals affected are totally asymptomatic and never realize that they have contracted an infectious disease. Three routes of transmission are known to exist.

1. Either the ingestion of cysts in raw or improperly cooked meat from domestic animals, such as cattle, sheep or pigs, or the ingestion of oocyst passed in cat feces.
2. Congenital transmission of the organism from the mother to the fetus across the placenta.
3. Infections acquired via a therapeutic procedure, such as blood transfusion or an organ transplant.

### ***Ingestion***

By and large, the overwhelming number of infections are acquired by the ingestion of cysts in improperly cooked meat. Ingestion of oocysts on vegetables contaminated with cat feces, vegetables eaten raw and not adequately washed, is another way in which a person could become infected. However, this route of infection is not considered a major contributor to the spread of this infection. The incidence of transmission from household pets has been scrutinized thoroughly, and although the debate is ongoing, most authorities feel there is little risk of transmission by this means if good personal and environmental sanitation is maintained.

### ***Congenital transmission***

The second mode of infection involves the congenital transmission of the organism to the fetus of a woman who acquires toxoplasmosis during pregnancy. This mode of infection can have serious consequences for the fetus, especially when infection occurs in the first trimester of pregnancy. It is widely believed that a previous (now inactive) infection carries no risk for the fetus.

***Blood transfusions or transplants***

Third and much less common, infection may be acquired by way of blood transfusions or organ transplants. However, the illness produced from infection acquired in this fashion may be much more virulent because the patient was already weakened by an underlying disease or may have had his or her immune system suppressed in order to accommodate a transplant procedure.

**Pathology and clinical manifestations**

Most *T. gondii* infections remain asymptomatic, but symptoms are much more likely to appear in individuals in whom the organism becomes disseminated passing through the epithelium of the intestine and multiplying in extraintestinal tissues. Inflammatory lesions form in the tissues of the organs infected. Most common of these include the reticuloendothelial system (mononuclear phagocytic system) (lymph nodes, liver, spleen) and the lungs, heart, brain, and eyes. The most common symptom of immunocompetent individuals is referred to as localized lymphadenopathy (regional lymph node enlargement), especially in the cervical region. Other symptoms accompanying this condition include fever, malaise, hepatosplenomegaly, and headache, making the misdiagnosis of this disease as mononucleosis or flu highly likely. The microscopic examination of the peripheral blood may only serve to confirm this misdiagnosis, since an absolute increase of lymphs with accompanying atypical lymphocytes will be observed. Occasionally, an immunocompetent patient may progress to a more serious form of this disease experiencing complications such as hepatitis, myocarditis, pneumonia, or encephalitis. In AIDS patients, toxoplasmotic encephalitis is the most common opportunistic parasitic infection of the central nervous system.

***Congenital toxoplasmosis***

Congenital toxoplasmosis may be totally asymptomatic or cause severe complications that commonly lead to stillbirths or that damage the central nervous system and eyes. The severity of the effects of the infections has been related to when, in the course of the pregnancy, the fetus acquired the infection. The general rule is that the earlier in pregnancy the fetus is infected, the more serious the results. Severe complications include brain damage with intracerebral calcification, hydrocephaly, microcephaly, chorioretinitis, fever, jaundice, rash, hepatosplenomegaly, xanthochromic CSF, and convulsions evident at birth or shortly thereafter. In children who survive infection, there is often congenital damage to the brain, manifested as mental retardation and epileptic seizures. Toxoplasmosis is a major cause of human birth defects.

***Other symptoms***

Asymptomatic infections can rapidly become acute infections with serious consequences to the patient if such immunosuppressive drugs as corticosteroids are administered. These infections may have been recently acquired or the infection may have been acquired years earlier and progressed asymptotically to a latent stage. In this latent form of the disease, the multiplication of the organism in extraintestinal tissues slows as the host immune cells, especially the T-lymphocytes, respond to the infection. This results in the accumulation of large numbers of organisms in infected host cells. These cells eventually become encapsulated within structures known as *pseudocysts* or *tachyzoites*. These generally remain dormant unless the host immune system weakens. The therapeutic suppression of the immune system in order to accommodate a transplant procedure, or the loss of immunocompetency from the effects of the AIDS virus, can be disastrous for a patient unwittingly harboring a latent toxoplasmosis infection. The renewed vigor of the organism usually expresses itself in the form of encephalitis, which occurs in approximately 50 percent of these patients and accounts for 90 percent of the fatalities. Other complications include the development of pneumonia or myocarditis.

**Laboratory diagnosis**

Specific diagnosis in humans is based on one or more laboratory tests. Immunological tests for toxoplasmosis are of greatest significance. However, there are four approaches available:

1. Direct examination.
2. Isolation of the organisms.
3. Examination for a presumptive diagnosis.
4. Immunological diagnosis.

#### ***Direct examination***

Demonstrating the organism in biopsy material is definitive. Direct examination impression films of suspected tissues or fluids should be air dried and stained with Giemsa stain for direct examination. The preparations usually examined are tissues taken by biopsy, sputum, vaginal exudates, and the sediment of spinal, pleural, or peritoneal fluids.

#### ***Isolation of organisms***

A variety of specimens are inoculated intraperitoneally into groups of white mice that have been dye-tested to make certain that they are free from infection. The diagnosis is confirmed by demonstration of cysts in the brains of the inoculated mice. Culture has proved to be very difficult.

#### ***Presumptive diagnosis***

The toxoplasmin skin test is widely employed in mass surveys to demonstrate *Toxoplasma* antibody, but it is not reliable because of cross-reaction with other antigens.

#### ***Immunological diagnosis***

The Sabin-Feldman dye test was the original standard procedure. The dye test involves working with live *T. gondii* organisms, but it is very sensitive and demonstrates specific antibody to *Toxoplasma*. Complement fixation tests, indirect hemagglutination tests, and indirect immunofluorescent tests are all available. At this time, the immunofluorescent test has gained in popularity since it does not require you to work with live organisms and designed to detect IgM antibodies, which is an advantage that is explained in the following paragraphs.

### **631. *Pneumocystis jiroveci* (formerly *Pneumocystis carinii*)**

*Pneumocystis jiroveci* (pronounced “yee row vet zee”) is a parasitic organism that was formerly classified as a protozoan. At the time of this writing, most current literature suggests that it should be reclassified with the fungi. It is accepted as fungus based on the following:

1. The ultrastructure of the cyst wall is similar to the cell wall of the fungi.
2. The lamellar cristae in the mitochondria is consistent with the fungi, protozoans have a tubular cristae.
3. The formation of intracystic bodies resembles the formation of ascospores by the Ascomycetes.
4. It has the highest homology of the more conserved domains of the 16S rRNA subunit with the Ascomycetes.
5. It has the homology of the 5S rRNA with that of primitive Zygomycetes.
6. Organism has the homology of the protein synthesis elongation factor EF-3 with that of *Saccharomyces cerevisiae* an Ascomycetes.
7. Separate proteins exist for thymidylate synthase and dihydrofolate reductase protozoa produce a singly bifunctional protein.
8. The organism has the homology of the sequence encoding *Pneumocystis* thymidylate synthase with that of Ascomycetes.

#### **Historical epidemiology**

Historically, this organism has usually been associated with outbreaks of plasma cell pneumonia in debilitated or immunosuppressed children found in hospitals, nurseries, or foundling homes in Europe



between the 1920s and 1950s. More recently, it has become an organism of major concern to immunosuppressed patients, the chronically ill, and those suffering from AIDS.

### Life cycle

How individuals are infected with this organism is yet unclear, but animal-to-animal transmission via the airborne route has been demonstrated in rats. It is theorized that subclinical infection with this organism is very common in the first years of life. In one study, approximately 75 percent of normal individuals were reported to have humoral antibody to *P. jiroveci* (formerly *carinii*) by the age of four. Such evidence suggests that subclinical infections are common, and that the organism then enters a latent phase in the tissues as long as the patient is not immunologically compromised. There are two known stages of this organism: a cyst and trophic form. The cyst, in the resistant stage, is 5 to 8  $\mu\text{m}$  in diameter and contains eight oval-shaped, intracystic bodies. Additionally, there is a pleomorphic, trophic form of this organism measuring 1 to 6  $\mu\text{m}$  in length, covered with tube-like projections that aid in the organisms attachment to epithelial cells. It is *assumed* that most individuals become infected when they inhale the cyst stage of the organism. The cyst then releases the intracystic bodies, which multiply asexually on the surfaces of epithelial cells. It is proposed that there is also a sexual reproduction cycle, which is by meiosis, however the mechanism is still not clear.

### Causal Agent

*Pneumocystis jiroveci* (previously classified as *Pneumocystis carinii*) was previously classified as a protozoa. Currently, it is considered a fungus based on nucleic acid and biochemical analysis. Refer to figure 5-4 for the following references. This is a generalized life cycle proposed by John J. Ruffolo, Ph.D. (Cushion, MT, 1988) for the various species of *Pneumocystis*. These fungi are found in the lungs of mammals where they reside without causing overt infection until the host's immune system becomes debilitated. Then, an oftentimes lethal pneumonia can result. Asexual phase: trophic forms ① replicate by mitosis ② to ③. Sexual phase: haploid trophic forms conjugate ① and produce a zygote or sporocyte (early cyst) ②. The zygote undergoes meiosis and subsequent mitosis to produce eight haploid nuclei (late phase cyst) ③. Spores exhibit different shapes (such as, spherical and elongated forms). It is postulated that elongation of the spores precedes release from the spore case. It is believed that the release occurs through a rent in the cell wall. After release, the empty spore case usually collapses, but retains some residual cytoplasm ④. A trophic stage, where the organisms probably multiply by binary fission is also recognized to exist. The organism causes disease in immunosuppressed individuals.

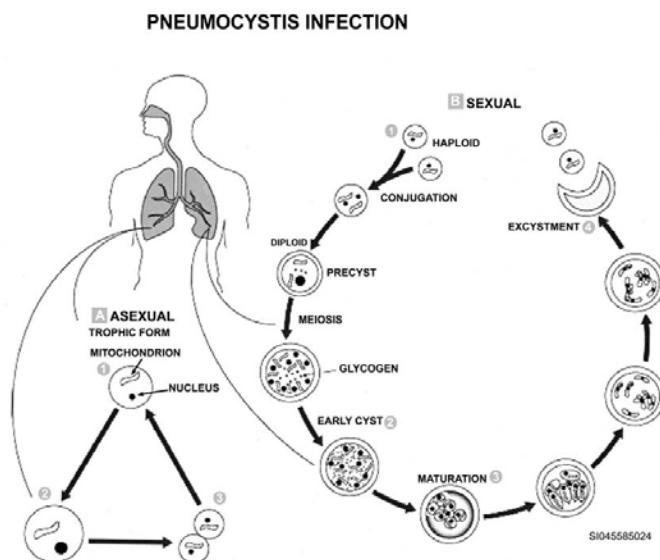


Figure 5-4. Life cycle of *Pneumocystis jiroveci*.

**Geographic Distribution**

Worldwide, in humans and animals. Serologic evidence indicates that most healthy children have been exposed by age 3 to 4. *Pneumocystis* pneumonia (PCP) occurs in immunosuppressed individuals and in premature, malnourished infants.

**Epidemiology**

This organism is distributed worldwide and is commonly found in the lungs of a variety of mammals (especially immunodeficient ones), including humans, primates, rodents, hares, ferrets, cats, dogs, and horses. Currently, the natural reservoir is unknown. *P. jiroveci* is the etiologic agent of two distinct clinical forms of pneumocystosis in humans: an epidemic and a sporadic. In its epidemic form, it causes plasma cell pneumonia in debilitated or premature infants between 3 to 6 months of age who have severe IgG and IgA deficiencies. Second, it is seen sporadically in hyperimmune pneumocystosis in children and adults who have received prolonged antibiotic or immunosuppressive therapy and in AIDS patients.

**Pathology and clinical manifestations**

Pneumocystosis is the most common cause of nonbacterial pneumonia in immunocompromised hosts in the United States. It is perhaps the single most important opportunistic infection of patients with AIDS, leading to high rates of morbidity and mortality. The most common clinical manifestations are dyspnea, fever, nonproductive cough, cyanosis, rales, and hepatosplenomegaly. The sporadic form of the disease can be fulminating and rapidly fatal; however, with appropriate and timely antibiotic treatment, pneumocystosis is curable in most cases. This disease primarily affects the lung, but can invade other organs.

**Laboratory diagnosis**

The best specimen is an open lung biopsy, but alternative specimens can yield a diagnosis. These alternate specimens include transthoracic lung aspirates, transbronchial biopsies, or occasionally bronchial washings. Sputum examination is not likely to reveal any disease process and most experts refuse to examine them. Impression smears of tissue are often faster and allow study of the internal cyst morphology. Cysts are 5 to 8  $\mu\text{m}$  in diameter and trophozoites are 1 to 6  $\mu\text{m}$ . It is important to differentiate these organisms from yeast cells. Methenamine silver (Gomori's) stains the cyst wall and does not stain trophozoites. If organisms are numerous, methenamine silver stains show non-budding cysts that are often cup shaped and, frequently, have a darker-staining central area.

**Other tests**

Immunodiagnostic tests for detecting antibody and antigen have been described but are of little assistance clinically because of lack of sensitivity and specificity. Calcofluor white chemofluorescent stain is a simple, rapid, and inexpensive method that has promise. Both DNA and RNA amplification procedures revealed high specificity and sensitivity, but are not practical for most clinical laboratories.

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**Self-Test Questions**

After you complete these questions, you may check your answers at the end of the unit.

**627. Genus *Plasmodia***

1. The reproductive cycle of the malarial parasite is similar to that of other members of what class?
2. Where does the asexual life cycle of the malarial parasite take place?

3. The disease malaria is started in humans as a result of being bitten by what type of insect?
4. The malarial parasite begins the exoerythrocytic stage in what organ?
5. During the asexual reproductive cycle of the malarial parasite, what develops as a result of the schizonts?
6. How do you refer to the series of events that goes on within a malarial infected cell?
7. What portion of the hemoglobin is used by a malarial parasite and which portion is not used?
8. What is created by the union of a microgamete and an oocyte?
9. What are four species of malarial parasite that infect man?
10. What are three factors that limit the prevalence of malaria?
11. What are two examples of genetic resistance to *P. falciparum*?
12. Which phase of the malarial parasite life cycle causes the major pathologic and clinical manifestations of malaria?
13. What is the most serious form of malaria?
14. What species of malarial parasites prefer to infect younger erythrocytes? Older?
15. What symptoms are indicative of the virulence of *P. falciparum*?

**628. Diagnosis of Plasmodia**

1. What is the term used to describe the disease caused by *P. vivax*?
2. In the type of malaria caused by *P. vivax*, at what intervals do fever spikes occur?

3. How do you describe the young trophozoite (ring form) of *P. vivax*?
4. What are the fine yellow-brown granules found in the growing trophozoite of *P. vivax*?
5. How many merozoites are found in a mature schizont (segmenter) of *P. vivax*?
6. What is the usual shape of a microgametocyte of *P. vivax* when it is fully mature?
7. What names have been given to the disease produced by *P. falciparum*?
8. What are the only forms of *P. falciparum* normally found in the peripheral blood?
9. How do you describe the shape and appearance of a macrogametocyte of *P. falciparum*?
10. What term is used to denote an infection with *P. malariae*?
11. What shape does the cytoplasm of a trophozoite of *P. malariae* assume within the red blood cell?
12. How many merozoites does a mature schizont of *P. malariae* contain?
13. What term is used to describe the arrangement of the merozoites in a mature schizont of *P. malariae*?
14. *Plasmodium ovale* is almost completely limited to what geographical region?
15. The erythrocytic cycle requires how many hours?
16. Red blood cells infected with *P. ovale* will show more of what type of dots over those infected with *P. vivax*?
17. How may the gametocytes of *P. malariae* and *P. ovale* be differentiated in thin-blood films?

**629. *Babesia* species**

1. In what two ways does the life cycle of *Babesia* species differ from the life cycle of the malarial parasite?
2. What is the most common form of the *Babesia* parasite found in humans?
3. How may a *Babesia* infection persist even in the absence of a vertebrate host?
4. In what part of the United States have most cases of babesiosis been diagnosed?
5. In which individuals is the severity of babesiosis more marked?
6. What three methods are available for the diagnosis of babesiosis?

**630. *Toxoplasma gondii***

1. What is the definitive host of *T. gondii*?
2. How many stages does *T. gondii* have in its life cycle, and where does the first stage take place?
3. What are the steps in the asexual cycle of *T. gondii*?
4. What characterizes the second stage of the life cycle of *T. gondii*?
5. In what type of climate is *T. gondii* most prevalent?
6. What are three routes of transmission of *T. gondii*?
7. What route of transmission of *T. gondii* is the most common?
8. Acquiring toxoplasmosis during what trimester of pregnancy is most likely to have serious consequences for the fetus?

9. In what tissues are inflammatory lesions due to a toxoplasmosis infection most likely to occur?
10. What is the most common symptom exhibited by an immunocompetent individual who contracts toxoplasmosis?
11. What evidence from the microscopic examination of peripheral blood may serve to confirm a misdiagnosis of mononucleosis in a patient with toxoplasmosis?
12. The severity of the effects of a congenial toxoplasmosis is determined by what factor?
13. How does *T. gondii* usually express itself in a patient who has had a dormant infection and receives immunosuppressive drugs?
14. What type of test is of greatest significance in diagnosing *T. gondii*?

**631. *Pneumocystis jiroveci* (formerly *carinii*)**

1. What type of epidemiology was historically associated with *Pneumocystis jiroveci* (formerly *carinii*)?
2. How are individuals thought to contract *P. jiroveci* (formerly *carinii*)?
3. When do many individuals experience subclinical infections with *P. jiroveci* (formerly *carinii*)?
4. What are the two known stages of this organism?
5. How and where does *P. jiroveci* (formerly *carinii*) multiply?
6. What are the two distinct clinical forms of pneumocystosis in man?
7. What are the most common clinical manifestations of pneumocystosis?

8. Pneumocystosis primarily affects what organs of the body?
9. What is the best specimen for use in diagnosing pneumocystosis?

## 5-2. The Hemoflagellates

The trypanosomes live in human blood, body fluids, and tissues, and the *Leishmania* species are considered intracellular parasites that live on macrophages of the skin, subcutaneous tissues, liver, spleen, bone marrow, and lymph nodes. African trypanosomiasis (*Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*) is also known as African sleeping sickness. *T. cruzi* causes Chagas disease or American trypanosomiasis that is seen in the New World. *T. rangeli* is a new species found in Central and South America. The *Leishmania* species cause Old World cutaneous, New World cutaneous, mucocutaneous, and visceral leishmaniasis in humans. The organisms mentioned above, as well as others, are discussed in this section. as they are discussed by Klaas in *Clinical And Pathogenic Microbiology*.

### 632. *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*

East African (*T. brucei rhodesiense*) trypanosomiasis has a more rapid disease progression than that of the West African form (*T. brucei gambiense*).

#### Life cycle

Refer to figure 5-4 as you study the life cycle of these organisms.

There are two subspecies of *Trypanosoma brucei*, *rhodesiense* and *gambiense*, and both are causative agents of African trypanosomiasis (African sleeping sickness). These organisms are hemoflagellates. There are slight differences in the insect vectors and animal reservoirs between the two species. Species of the tsetse fly transmit both forms. The fly takes a blood meal and transfers the trypomastigotes into the bite wound from its salivary ducts. The flies are usually infective for their entire lifespan. Development of the infective stage within the fly requires 25 to 50 days, depending on the species of fly, the strain of parasite, and the ambient temperature. The infective trypomastigotes are slender, spindle-shaped flagellates (15 µm long), with an undulating membrane extending the full length of the body. After entry through the skin, the trypomastigotes transform into elongate, more slender forms reproducing asexually for 1 or 2 days before entering the peripheral blood and lymphatic circulation. In the peripheral circulation, the trypomastigotes continue to divide extracellularly and take a variety of shapes that can be observed in blood films. Usually long, slender trypomastigotes about 28 µm long with a free flagellum extending from the anterior terminus of the undulating membrane are observed. Short (15 µm) aflagellate forms are less common but may be observed.

#### Causal Agents

Protozoan hemoflagellates belonging to the complex *Trypanosoma brucei*. Two subspecies that are morphologically indistinguishable cause distinct disease patterns in humans: *T. b. gambiense* causes West African sleeping sickness and *T. b. rhodesiense* causes East African sleeping sickness. (A third member of the complex, *T. b. brucei*, under normal conditions does not infect humans).

Refer to figure 5-5 for the following references. During a blood meal on the mammalian host, an infected tsetse fly (genus *Glossina*) injects metacyclic trypomastigotes into skin tissue. The parasites enter the lymphatic system and pass into the bloodstream ❶. Inside the host, they transform into bloodstream trypomastigotes ❷, are carried to other sites throughout the body, reach other blood

fluids (e.g., lymph, spinal fluid) and continue the replication by binary fission <sup>3</sup>. The entire life cycle of African Trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host (<sup>4</sup>, <sup>5</sup>). In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission <sup>6</sup>, leave the midgut, and transform into epimastigotes <sup>7</sup>. The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission <sup>8</sup>. The cycle in the fly takes approximately 3 weeks. Humans are the main reservoir for *Trypanosoma brucei gambiense*, but this species can also be found in animals. Wild game animals are the main reservoir of *T. b. rhodesiense*.

### Geographic Distribution

*T. b. gambiense* is found in foci in large areas of West and Central Africa. The distribution of *T. b. rhodesiense* is much more limited, with the species found in East and Southeast Africa.

### Epidemiology

The distribution of African trypanosomiasis is limited by the natural range of the insect vector and covers large swath of tropical regions of Africa, from roughly 15°N to 15° to 25°S. Within this geographic region the range of the two vectors and two forms of the disease overlap. The Gambian form of the disease is more prevalent. The Rhodesian form is seen more sporadically. There have been isolated reports of the disease being transmitted via blood transfusions.

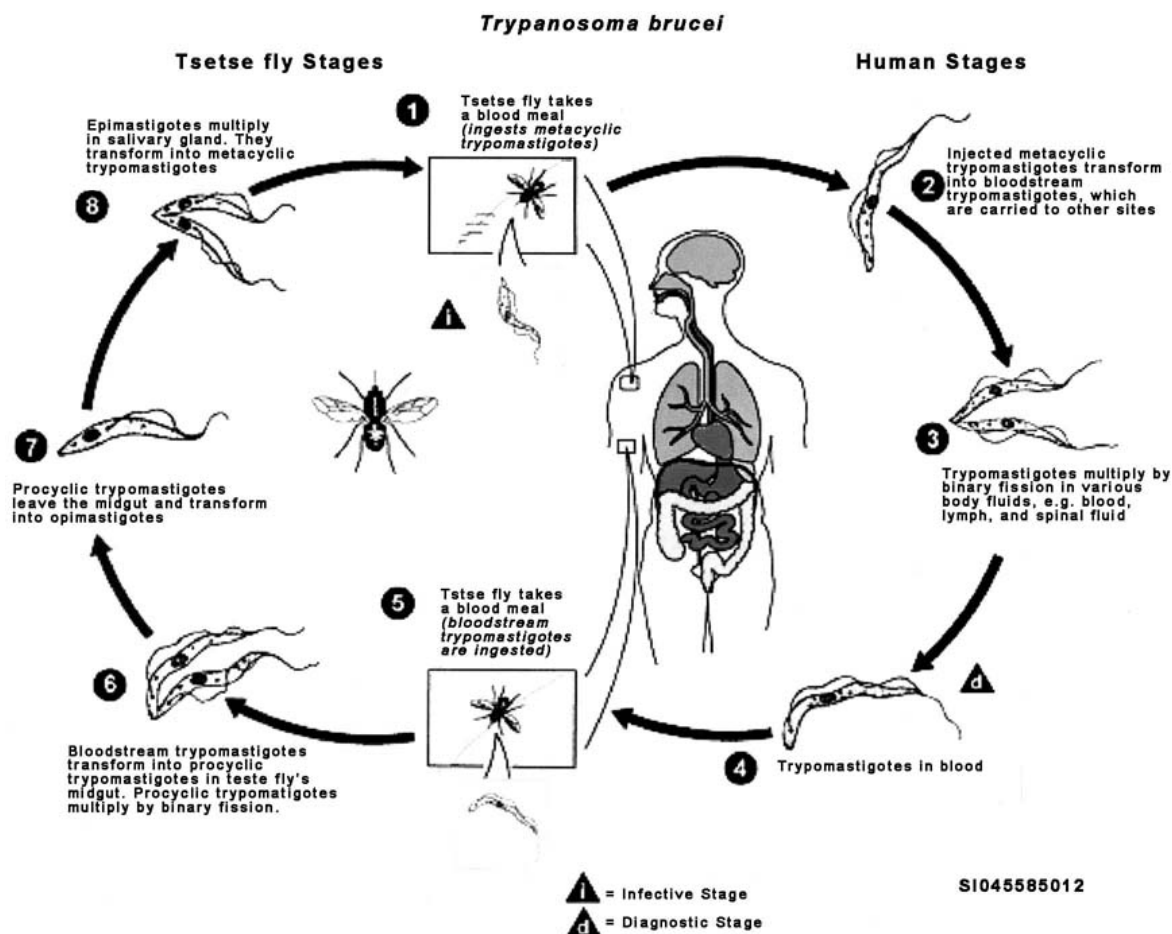


Figure 5-5. Life cycle *Trypanosoma brucei*.



### Pathology and clinical manifestations

The two diseases have nearly the same pathologic effects. A hard and painful nodule or chancre will develop within the first two weeks at the site of the fly bite. After the parasites begin their transformation and reproduction and invade the bloodstream the chancre will disappear. The patient will appear seemingly healthy at this stage. Rarely, the infection fails to progress beyond this stage in some patients. In most cases the infection progresses whereby the trypanosomes invade the reticuloendothelial system adversely affecting lymph tissue and causing intermittent febrile attacks. The attacks last one to seven days and are accompanied by high, irregular fever; malaise; night sweats; and headaches. A symptom free period follows these attacks that can last several weeks. Interestingly, the subsequent attacks feature distinct and separate subgroups of the trypanosomal parasites that possess slightly different surface antigens thus defeating the host's immune response. This causes a generalized lymphadenopathy, especially by an enlargement of the lymph nodes in the posterior cervical triangle (Winterbottom's sign). Each successive attack weakens the immune response until it is overwhelmed impacting the lymph system and then the central nervous system. At this stage, many mental syndromes will be prevalent including personality changes, dementia, hysteria and delusions, to name a few. In final stages of the infection, the patient develops an uncontrollable desire to sleep, severe malnutrition, and loss of motor control. The patient will lapse into a coma before death if they do not succumb to malnutrition, or a secondary bacterial infection first.

### Laboratory diagnosis

Basic laboratory procedures cannot differentiate between the two subspecies of this organism. Speciation results from the clinical picture and geographic region where the infection was acquired. The role of the laboratory is to identify and confirm the presence of the trypomastigotes and assist the provider in determining the stage of infection (bloodstream, lymphatics, central nervous system). In the early stages of infection, the parasites may be detected in smears of the peripheral blood or from lymph node aspirates. Typically, peripheral blood samples are better for detecting the Rhodesian form of the disease; whereas lymph node aspirates are more likely to reveal the Gambian form. The trypanosomes may be detected in spinal fluid when there is central nervous system involvement. Wet mounts may be used to detect the parasites whip-like motility through a mass of red cells however this does not negate the need to perform thick and thin Giemsa stained blood film examinations. In stained smears, the trypomastigotes of both subspecies appear as spindle-shaped flagellates of varying length (12 to 40  $\mu\text{m}$ , average 25  $\mu\text{m}$ ). An undulating membrane, originating in a small, red-staining kinetoplast near the rounded posterior end, extends the entire length of the body and terminates in a single free flagellum at the pointed anterior end are all keys to identifying this parasite.

### 633. *Trypanosoma cruzi*

American trypanosomiasis infections caused by *T. cruzi* can also be very serious with high morbidity and mortality. As you will learn in the following text, this organism is spread by the insect vector the reduviid bug, which is also known as the kissing bug, and also as the assassin bug.

### Life cycle

Refer to figure 5–6 as you study the life cycle of these organisms.

*Trypanosoma cruzi* is the causative agent of American trypanosomiasis (Chagas' disease). It differs from the African variety of trypanosomiasis in that it has an intracellular aflagellate stage known as an amastigote instead of a circulating trypomastigote. This aflagellate stage is the reproductive stage of the parasite and is for the pathogenic and clinical manifestations of the disease. The trypomastigotes are short with an undulating membrane and a flagellum. They are introduced onto the host's skin via the feces of the insects of the family Reduviidae (genus *Rhodnius*, *Triatoma*, or *Panstrongylus*). The insect defecates when it takes a blood meal from the host, and the infection is introduced when the host scratches the bite site spreading the feces into the wound, and subsequently the bloodstream.

### Causal Agent

The protozoan parasite, *Trypanosoma cruzi*, causes Chagas disease, a zoonotic disease that can be transmitted to humans by blood-sucking triatomine bugs.

An infected triatomine insect vector (or “kissing” bug) takes a blood meal and releases trypomastigotes in its feces near the site of the bite wound. Trypomastigotes enter the host through the wound or through intact mucosal membranes, such as the conjunctiva **1**. Common triatomine vector species for trypanosomiasis belong to the genera *Triatoma*, *Rhodnius*, and *Panstrongylus*. Inside the host, the trypomastigotes invade cells, where they differentiate into intracellular amastigotes **2**. The amastigotes multiply by binary fission **3** and differentiate into trypomastigotes, and then are released into the circulation as bloodstream trypomastigotes **4**. Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle. The bloodstream trypomastigotes do not replicate (different from the African trypanosomes). Replication resumes only when the parasites enter another cell or are ingested by another vector. The “kissing” bug becomes infected by feeding on human or animal blood that contains circulating parasites **5**. The ingested trypomastigotes transform into epimastigotes in the vector’s midgut **6**. The parasites multiply and differentiate in the midgut **7** and differentiate into infective metacyclic trypomastigotes in the hindgut **8**.

*Trypanosoma cruzi* can also be transmitted through blood transfusions, organ transplantation, transplacentally, and in laboratory accidents.

### Geographic Distribution

The Americas from the southern United States to southern Argentina. Mostly in poor, rural areas of Central and South America. Chronic Chagas disease is a major health problem in many Latin American countries. With increased population movements, the possibility of transmission by blood transfusion has become more substantial in the United States.

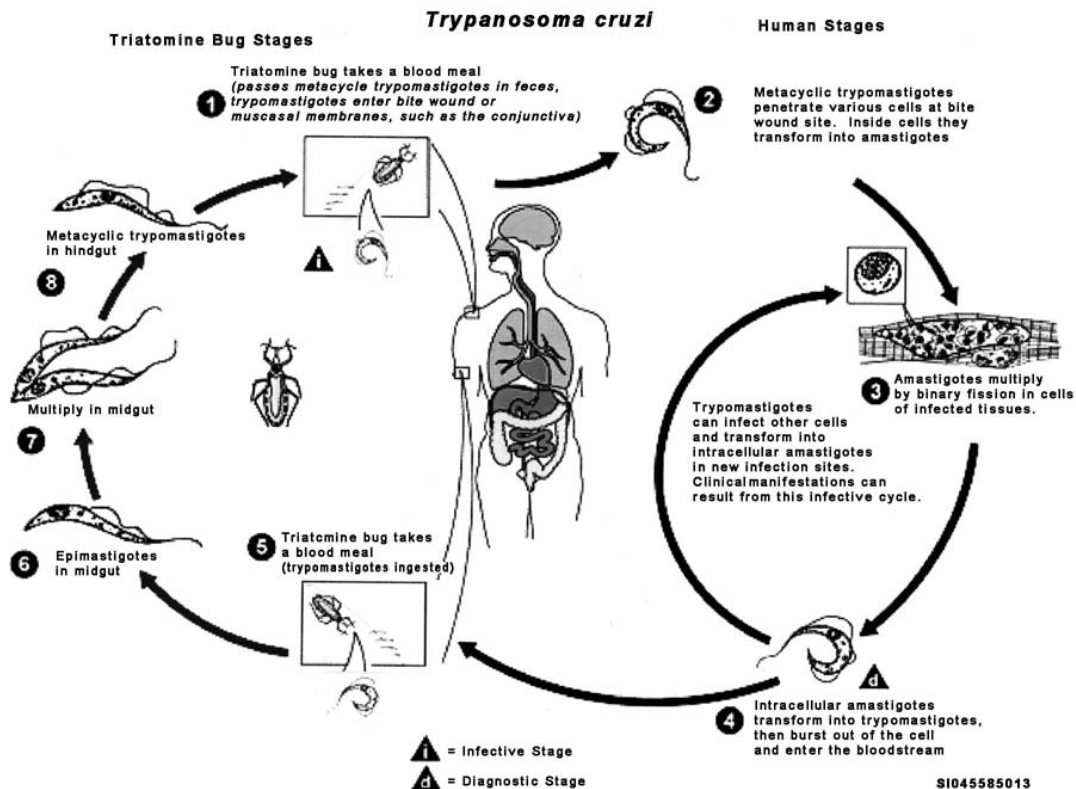


Figure 5-6. Life cycle *Trypanosoma cruzi*.

Once the trypomastigotes penetrate the mucous membranes they enter a variety of cells, most notably fixed phagocytic cells. In these cells the trypomastigotes transform into the small round amastigote form. Asexual reproduction occurs during this stage. Within three to four days rupture of the infected cells is followed by the release of daughter amastigotes that spread to infect other cells or form trypomastigotes that circulate in the peripheral blood. In the latter stages of the disease, the spreading amastigotes infect the lymph nodes which is followed by the infestation of nearly all the body's organs especially cells of the reticuloendothelial system, the muscle cells of the heart, and the neurological cells of the central nervous system. In the late stages of the disease, there are only small numbers of trypomastigotes in the peripheral blood stream. Insect vectors ingest these trypomastigotes where they transform into an epimastigote in the midgut of the insect. The epimastigote has the kinetoplast located anteriorly to the nucleus, an undulating membrane that extends half the length of the body and a free flagellum. The epimastigotes multiply asexually in the posterior midgut. Under proper conditions, they become infective and migrate to the hindgut in eight to ten days.

### **Epidemiology**

The vector for American trypanosomiasis is widespread in the Americas and infects many wild and domestic animals, mainly rodents, dogs and cats. The parasite is most prevalent in South America, especially the tropical countries. It is less prevalent in Central and North America. In the United States, the parasite is found in states along the southern tier. The vast majority of cases in the United States are found in immigrants or travelers from endemic countries. This is mostly because of the differences in feeding habits of the vector and housing design that more readily allows human contact with the vector. The most important factor in determining disease distribution is differences in housing conditions of populations at risk of acquiring the disease. Those living in poorly constructed dwellings usually made of mud or other raw unprocessed materials are most at risk and usually seen more in rural areas as opposed to urban areas where housing construction is more substantial. These raw materials are akin to the natural habitat of the vector. Even in geographical regions where the parasite is widely dispersed, the greatest impact of the disease is typically seen in the rural areas as opposed to urbanized areas of the region. Other potential modes of transmission include domesticated animals and household pets and through blood via transfusion or illicit drug use by reusing hypodermic needles.

### **Pathology and clinical manifestations**

The vector for this disease has an affinity to often bite the face of the victim, allowing for the moniker – “kissing bug.” The disease can manifest in two phases: acute and chronic. The acute phase lasts for one to four months after infection. The chronic phase may not appear until decades after infection. Usually the acute phase afflicts children under two years of age. It produces facial swelling and drastic edema of the eyelids of one eye when the parasite enters through the eye through rubbing. A less common manifestation is when the parasite enters through a skin abrasion causing erythema and swelling known as a chagoma. Chagomas can appear anywhere on the body but are most common on the face. There are many symptoms associated with American trypanosomiasis, most are nondescript and mainly associated with the host's inflammatory response and destruction of the parasitized cells including fever, headache, malaise, muscle pain, generalized lymphadenopathy, and hepatosplenomegaly. Most commonly however, American trypanosomiasis results in the development of cardiac abnormalities. This may manifest with tachycardia or with symptoms that require detailed examinations for detection. The majority of the deaths caused by this disease in the acute phase are from these cardiac abnormalities and meningoencephalitis. Five to ten percent of deaths that occur in the acute phase of the disease are most likely to affect children. The disease enters a chronic phase following the acute phase where there is a slow but continual cellular dysfunction especially the nerves and muscle cells of hollow organs. Typical organs affected include the heart, esophagus and intestines. As the disease progresses, the heart becomes enlarged and flabby

to the point that it is no longer sufficient to function. The decreased peristalsis and massive distention caused by the loss of muscle tone in the digestive tract is termed megadisease.

### **Laboratory diagnosis**

The laboratory techniques used to diagnose a *T. cruzi* infection depends on the phase of the disease. In the acute phase the parasites are abundant in the peripheral blood and may be detected with a wet mount as well as thick and thin Giemsa stained blood films. The trypomastigotes are difficult to see in wet mounts but their forward whip like motion through the blood cells is easily detected.

Confirmation is via detection of the parasite on thick and thin Giemsa stained blood films. Much like the African variety, the trypomastigotes exhibit a 15 to 20  $\mu\text{m}$  U- or C-shaped body. The microscopic features of the organism are a large kinetoplast in the pointed posterior end of the body and an undulating membrane that runs the full length of the body, where it terminates in a single, free flagellum. Confirmation with Giemsa stained smears is critical because a non-pathogenic trypanosome known as *T. rangeli* can cause confusion towards a diagnosis. The trypomastigotes of *T. rangeli* are easily distinguished from *T. cruzi* as they have a smaller kinetoplast and are almost double its length and do not exhibit a C or U shape. One of the most interesting differences between American and African trypanosomiasis is that in the American variety total IgM remains within normal limits unlike the spike seen in the African variety.

In the chronic phase of the disease, the trypanosomes become less distributed in the peripheral blood and require specialized techniques for detection. One of the best techniques that is available to most clinical laboratories is lysis centrifugation. Anticoagulated blood is collected and the erythrocytes are lysed and centrifuged and the sediment is smeared and stained as if it were a blood smear. More advanced laboratories utilize more specialized techniques such as in vitro or in vivo cultivation. Xenodiagnosis is used mainly in endemic regions and is very sensitive. In this technique, disease free laboratory-reared reduviids feed on the patient and, after 30 to 60 days, examined for metacyclic trypomastigotes in their rectal contents. Serologic methods for detection exist but have limited success.

### **634. *Leishmania tropica* complex, *Leishmania mexicana* complex, *Leishmania braziliensis* complex, and *Leishmania donovani* complex**

These obligate intracellular parasites are transmitted by bites of infected sand flies. Leishmaniasis is mainly a zoonosis, although humans serve as the reservoir in certain areas. More than 400,000 new cases are reported annually with approximately 12 million currently infected. *Leishmania* can serve as a prime example of the effect of a parasite on battlefield operations. During Operation Iraqi Freedom (OIF) which began in March 2003, a large number of troops became infected with cutaneous leishmaniasis. Troops typically are afflicted on exposed areas of the body (face, neck, forearms) and call it 'Baghdad boil.' Without treatment, the infection can develop large sores (about quarter sized) on these areas. The sores are not painful or contagious but in many cases, the sores can be disfiguring without treatment. There was a large increase in the number of leishmania infections seen during OIF in comparison to Operation Desert Storm. Approximately 20 infections were observed in Operation Desert Storm whereas one unit of nearly 750 saw more than 200 cases of leishmaniasis. Many of these troops were sent from the battlefield to Washington D.C. for treatment. If you were to apply these statistics to a more virulent and debilitating parasite, you can easily deduce the effect it might have on battlefield success, especially if one-third of a unit is off its feet and in a rear echelon receiving treatment. Thus, the importance of keeping a current and working knowledge of parasitic disease is critical not only for the patient, but for triumph on the battlefield by providing rapid diagnosis and returning these members to duty as soon as possible.

### **Life cycle**

The life cycles of all members of the genus *Leishmania* are identical. *Leishmania* is spread by the sand fly vector; *Lutzomyia* species in the western hemisphere and *Phlebotomus* in other endemic

areas of the world. Human infections begin when an infected female sand fly takes a blood meal and injects the promastigotes into the skin. Promastigotes have a single anterior flagellum and lack an undulating membrane. After injection into the skin phagocytic cells ingest them where they transform into aflagellate amastigotes. They proliferate in the phagocytic cells of the reticuloendothelial system and the endothelial cells of the capillaries. Depending on the type of leishmaniasis (dermotropic or viscerotropic) the disease will develop into cutaneous, mucocutaneous or visceral types of leishmaniasis. Typically, the type of leishmaniasis is determined by the primary location of the affected macrophages that are infected. The cutaneous form typically only affects the site of the bite, whereas the mucocutaneous will affect skin and mucous membranes widely throughout the body. The visceral form will affect a wide range of internal organs, notably preferring to multiply in the spleen, liver, and bone marrow. Female sand flies become infected when they feed on a host infected with the amastigote stage of the parasite. They develop and reproduce in the midgut of the fly. The developed promastigotes migrate to the upper digestive tract of the fly. The fly will remain infective during its entire two to three week lifespan.

### Causal Agent

Refer to figure 5-7 for the following discussion. Leishmaniasis is a vector-borne disease that is transmitted by sand flies and caused by obligate intracellular protozoa of the genus *Leishmania*. Human infection is caused by about 21 of 30 species that infect mammals. These include the *L. donovani* complex with 3 species (*L. donovani*, *L. infantum*, and *L. chagasi*); the *L. mexicana* complex with 3 main species (*L. mexicana*, *L. amazonensis*, and *L. venezuelensis*); *L. tropica*; *L. major*; *L. aethiopica*; and the subgenus *Viannia* with 4 main species (*L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis*, and *L. (V.) peruviana*). The different species are morphologically indistinguishable, but they can be differentiated by isoenzyme analysis, molecular methods, or monoclonal antibodies.

### LEISHMANIASIS

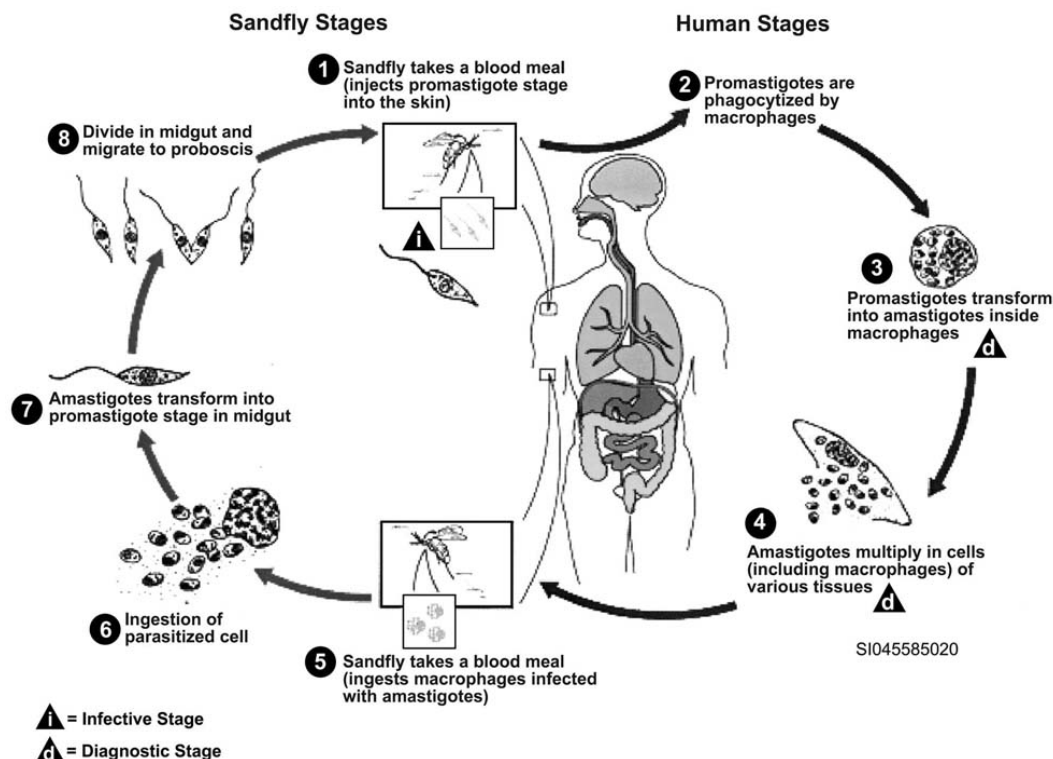


Figure 5-7. Life cycle of *Leishmania* species.

Leishmaniasis is transmitted by the bite of female phlebotomine sand flies. The sand flies inject the infective stage, promastigotes, during blood meals ❶. Promastigotes that reach the puncture wound are phagocytized by macrophages ❷ and transform into amastigotes ❸. Amastigotes multiply in infected cells and affect different tissues, depending in part on the *Leishmania* species ❹. This originates the clinical manifestations of leishmaniasis. Sand flies become infected during blood meals on an infected host when they ingest macrophages infected with amastigotes (❺, ❻). In the sand fly's midgut, the parasites differentiate into promastigotes ❼, which multiply and migrate to the proboscis ❽.

### Geographic Distribution

Leishmaniasis is found in parts of about 88 countries. Approximately 350 million people live in these areas. Most of the affected countries are in the tropics and subtropics. The settings in which leishmaniasis is found range from rain forests in Central and South America to deserts in West Asia. More than 90 percent of the world's cases of visceral leishmaniasis are in India, Bangladesh, Nepal, Sudan, and Brazil.

Leishmaniasis is found in Mexico, Central America, and South America—from northern Argentina to southern Texas (not in Uruguay, Chile, or Canada), southern Europe (leishmaniasis is not common in travelers to southern Europe), Asia (not Southeast Asia), the Middle East, and Africa (particularly East and North Africa, with some cases elsewhere).

### Epidemiology

Many subspecies of *Leishmania* exist and extensive DNA and similar studies reveal more all the time. Diagnosis and treatment of the infection is based upon differences in the clinical picture presented by the patient whether cutaneous, mucocutaneous, or visceral. Two major complexes cause cutaneous leishmaniasis *Leishmania tropica* complex and *Leishmania mexicana* complex. The former is responsible for Old World cutaneous leishmaniasis or Oriental sore. Members of this complex include *L. tropica major*, *L. tropica minor*, and *L. aethiopica* and are endemic to India, Africa, the Mediterranean basin and the Middle East. The latter is the causative agent of New World cutaneous leishmaniasis or chiclero ulcer. Members of this complex are *L. mexicana mexicana*, *L. mexicana pifanoi*, and *L. mexicana amazonensis* and are endemic to Central and South America. *Leishmania* infection is rare in the United States. Within these broad geographic regions there are differences in the parasite species, reservoir host and insect vector. Within these broad geographic areas, there are regional differences in the species of parasite, the reservoir host, and the insect vector. Visceral leishmaniasis (kala azar) is the most widely distributed form of leishmaniasis and considered endemic in the Mediterranean basin, India, East Africa, Central and South America, and China. Visceral leishmaniasis is essentially a disease of rural areas; however, as with cutaneous forms of leishmaniasis, regional differences exist in the reservoir host and insect vector.

### Pathology and clinical manifestations

In cutaneous leishmaniasis early lesions develop into single or sometimes multiple erythematous papules at the site of the sand fly bite. Amastigotes continue their reproduction within the skin. As this occurs, the papule increases in size and develops multiple satellite lesions in proximity of the primary lesion. In a few weeks, these lesions ulcerate which eventually heal with scarring. The clinical presentation of the disease will vary based on the species involved. Some of the most common presentations include that of the Old World leishmaniasis with its moist ulcers caused by *L. tropica major* and the dry ulcers associated with *L. tropica minor*. Onset of the disease with the moist ulcerative variety is rapid and will heal within six months. The dry ulcerative variety may have a long incubation period and a lesion that may not heal for several years as well. Healing of these cutaneous lesions usually represents lifelong immunity. Prior to the healing of the lesions, there is a risk of bacterial infections or development of a mucocutaneous infection.

Mucocutaneous leishmaniasis is caused by *L. braziliensis* occurring when the ulcer penetrates deep into the tissue. It may then spread to the bloodstream where it disseminates. It has an affinity for the tissues of the mucocutaneous borders and cartilaginous regions of the nose and mouth where it causes tissue destruction and deformity. Secondary bacterial infections in mucocutaneous leishmaniasis are common.

In visceral leishmaniasis, lesions may go undetected and *L. donovani* is the usual causative agent. The incubation period for this infection may last from under two weeks to nine years. There is typically a gradual onset with an irregular undulant fever, which later takes on a pattern that resembles brucellosis. The most common manifestations of the disease include splenomegaly, lymphadenopathy and hyperpigmentation of the skin of the hands, feet, and abdomen called *kala azar*. (The term *kala azar* can be translated as “black disease”).

In untreated or immunocompromised patients, jaundice or blood disorders will occur as well as a multitude of secondary infections. Once the disease reaches the terminal stage, it claims nearly 75 percent of its victims.

### **Laboratory diagnosis**

Giemsa stained touch preparations from the edges of active lesions are used to detect the amastigote for the diagnosis of cutaneous leishmaniasis. Detection during the early stages of mucocutaneous leishmaniasis also uses this technique. In the later stages of mucocutaneous leishmaniasis require in vitro cultivation of the promastigote in Novy, MacNeal Nicolle (NNN) medium. Keep in mind that the organism does not always grow well in vitro and diagnosis may rest on the Montenegro skin test along with the patient history and physical examination. Biopsy tissue material is required for the detection of visceral leishmaniasis, preferably from the spleen. Serologic procedures are also available. Many laboratories do not perform the tests required for diagnosis of these tropical diseases. Often they must report the non-specific ‘amastigote of hemoflagellate seen.’ This reporting method applies to the Trypanosomes as well. The laboratory should make every effort to consult with a reference laboratory to obtain a definitive diagnosis.

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## **Self-Test Questions**

**After you complete these questions, you may check your answers at the end of the unit.**

### **632. *Trypanosoma brucei* species *gambiense* and *Trypanosoma brucei* species *rhodesiense***

1. What are the major differences between the life cycles of *Trypanosoma brucei gambiense* and *rhodesiense*?
2. What is the vector of African trypanosomiasis?
3. What happens to the trypomastigotes once they are ingested by a tsetse fly during a blood meal?
4. What factors determine the length of time required to develop the infective stage of trypanosomes within the tsetse fly?
5. What factors limit the distribution of African trypanosomiasis?

6. Which species of *Trypanosoma* is generally more prevalent?
7. What differences exist between *T. brucei gambiense* and *rhodesiense* in the pathologic and clinical manifestations they produce?
8. What is usually the first sign of trypanosomiasis?
9. Once trypanosomes invade the reticuloendothelial system, what symptoms does the patient experience?
10. What characteristic of each subgroup of trypanosomes is responsible for the patient's febrile episodes?
11. What pathology marks the later stages of untreated African trypanosomiasis?
12. What are the major clinical differences between the two forms of African trypanosomiasis?
13. In general, what specimen is considered *better* for detecting the Rhodesian form of trypanosomiasis? The gambiae form?

**633. *Trypanosoma cruzi***

1. What is the most important difference between American and African trypanosomiasis?
2. What is the vector for *T. cruzi*?
3. How does the metacyclic trypomastigote of *T. cruzi* enter the human body?
4. Initially, where do the amastigotes of *T. cruzi* metastasize?



5. In the latter stages of a *T. cruzi* infection, what cells of the body are especially likely to be infected?
6. What form of the *T. cruzi* organism actively multiplies in the posterior region of the midgut of the insect vector by asexual division?
7. Besides humans, what other mammals are infected by *T. cruzi*?
8. What is thought to be the single most important factor determining the distribution of *T. cruzi*?
9. Besides natural transmission of *T. cruzi* by its insect vector, what other routes of infection exist?
10. How do you describe the symptoms of a *T. cruzi* infection in the acute stage in a child under 2 years of age?
11. What symptom develops if *T. cruzi* enters the body through an abrasion of the skin?
12. What is the most common manifestation of acute American trypanosomiasis?
13. What percentage of *T. cruzi* infections result in the death of the patient?
14. What are the *principal* manifestations of a chronic *T. cruzi* infection?
15. What is meant by the term *megadisease*?
16. What techniques would be appropriate for the recovery and identification of trypomastigotes in the acute early stage of a *T. cruzi* infection?

17. What microscopic morphology does *T. cruzi* present in thick and thin Giemsa-stained blood smears?
18. Why is it important to confirm the identification of *T. cruzi* with stained blood smears?
19. What serological clue in the form of antibody levels helps to distinguish American and African trypanosomiasis?
20. What is the best technique available to most clinical laboratories for demonstrating *T. cruzi* trypomastigotes in the later chronic stages of American trypanosomiasis?

**634. *Leishmania tropica* complex, *Leishmania mexicana* complex, *Leishmania braziliensis* complex, and *Leishmania donovani* complex**

1. What form of the leishmanial organism is infective for humans when injected into the skin as infected female sand flies take a blood meal?
2. What genera of sand flies act as the vector for *Leishmania*?
3. Where do the amastigote form of the *Leishmania* proliferate in the body?
4. Once formed, where do the amastigotes of various leishmanial organisms spread?
5. How do female sand flies become infected with leishmanial organisms?
6. What are the three major clinical forms of leishmaniasis?
7. What is the most widely distributed form of leishmaniasis?
8. What are two of the most common clinical patterns of Old World cutaneous leishmaniasis?
9. What does the healing of the lesions of cutaneous leishmaniasis usually signify?

10. What are two possible serious complications of cutaneous leishmaniasis?
11. What characteristics of the subspecies *L. braziliensis* makes infection with it very serious?
12. What is meant by the term *viscerotropic* when applied to *L. donovani*?
13. What are the earliest manifestations of visceral leishmaniasis?
14. What is the laboratory diagnosis of cutaneous leishmaniasis based upon?
15. What techniques may be necessary for the diagnosis of mucocutaneous leishmaniasis in the later stages of the disease?
16. What are the specimens of choice for diagnosing the later stages of visceral leishmaniasis?

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### Answers to Self-Test Questions

**627**

1. Sporozoea.
2. Humans.
3. An infected female *Anopheles* mosquito.
4. In the liver.
5. Daughter merozoites.
6. Erythrocytic stage.
7. Protein; iron.
8. Ookinete.
9. Vivax, malariae, falciparum, and ovale.
10. (1) The number of suitable host.  
(2) The presence of a suitable vector.  
(3) Local weather conditions.
11. S. hemoglobin and favism.
12. Erythrocytic phase.
13. *P. falciparum*.
14. *P. vivax* and *P. ovale*; *P. malariae*.
15. Thrombosis of the capillaries, ischemia, and tissue anoxia within affected organs.

**628**

1. "Tertian" malaria.
2. 48 hours intervals (or every third day).
3. It usually has a large chromatin dot and the cytoplasmic circle is large and pale blue in color.
4. Malarial pigment.
5. 12 to 24.
6. Circular or ovoid.
7. "Malignant tertian," or "estival-autumnal."
8. Ring forms and gametocytes.
9. Long, slender, sausage-shaped, with a concentrated mass of dark pigment near the center surrounding a dark red chromatin mass.
10. Quartan malaria.
11. A broad band.
12. 6 to 12.
13. Rosette or daisy form.
14. Parts of West Africa.
15. 48 hours.
16. Schüffner's.
17. The gametocytes of *P. ovale* will contain Schüffner's dots.

**629**

1. The absence of exoerythrocytic and sexual stages.
2. Intraerythrocytic trophozoites.
3. In addition to blood transfusions, the tick may transmit the infection from one stage to another, and from one generation to another.
4. Several islands off the coast of New England.
5. Individuals who are immunosuppressed, or who lack a spleen, and in the elderly.
6. (1) Thick and thin blood films stained with Giemsa stain.  
(2) Hamster inoculation.  
(3) Serological testing.

**630**

1. The domestic cat or other members of the family *Felidae*.
2. Two; in the epithelial cells of the small intestines.
3. (1) The ingestion of oocysts from the feces of another cat or in the tissues of a reservoir host such as rodents or birds, (2) the rupture of the oocysts and freeing of its sporozoites, which infect epithelial cells of the intestine where they are transformed into trophozoites, (3) the asexual division of the trophozoites (schizogony) and production of schizonts composed of many individual daughter cells called merozoites, and (4) the rupture of the cell and release of the merozoites.
4. Multiplication of trophozoites in cells outside the intestinal tract.
5. Warm moist climates.
6. Ingestion of cysts, congenital transmission, or via therapeutic procedure.
7. Ingestion of cysts in improperly cooked meat.
8. First trimester.
9. Reticuloendothelial system, lungs, heart, brain, and eyes.
10. Lymphadenopathy.
11. An absolute increase of lymphs with accompanying atypical lymphocytes.
12. At what stage of pregnancy the infection was acquired by the fetus.

13. Encephalitis.
14. Immunological.

**631**

1. Outbreaks of plasma cell pneumonia in debilitated or immunosuppressed children in hospitals, nurseries or foundling homes in Europe from the 1920s to 1950s.
2. Via an airborne route of infection when they inhale the cyst stage.
3. In the first years of life.
4. Cysts and trophic form.
5. Asexually; on the surface of epithelial cells.
6. Epidemic and sporadic.
7. Dyspnea, fever, nonproductive cough, cyanosis, rales, and hepatosplenomegaly.
8. The lungs.
9. Open lung biopsy.

**632**

1. The Rhodesian form has a slightly different species of insect vectors, a natural animal reservoir besides humans, and invades the central nervous system much more rapidly than the gambiae form.
2. Various species of tsetse flies.
3. They multiply in the midgut of the fly and then migrate to the fly's salivary glands, where they transform into epimastigotes.
4. The species of fly, strain of parasite, and ambient temperature.
5. The natural range of the insect vector.
6. Gambian form.
7. Except for differences in the rate at which the two diseases develop, both forms of African trypanosomiasis have nearly identical pathologic effects.
8. A hard, painful nodule or chancre that develops within 4 to 10 days at the site of the fly bite and is accompanied by regional lymphadenopathy.
9. Generalized hyperplasia of the lymphoid tissue and intermittent febrile episodes.
10. Febrile episodes are apparently initiated by separate subgroups of trypanosomes that have slightly different surface antigens.
11. Progressive inflammation of the brain, meninges and spinal cord.
12. The speed at which the disease develops and the extent of central nervous system involvement before death.
13. Peripheral blood samples. Lymph node aspirates.

**633**

1. In American trypanosomiasis, an intracellular, aflagellate stage (amastigote), rather than the circulating trypomastigote is the stage of the parasite that multiplies in the body.
2. Insect vectors of the family Reduviidae.
3. They are passed onto the host's skin in the feces of the vector and the trypomastigote may be rubbed into or actively migrate into the bite wound.
4. In the regional lymph nodes.
5. Fixed phagocytic cells of the reticuloendothelial system, the muscle cells of the heart, and the neuroglial cells of the central nervous system.
6. Epimastigote.
7. Opossums, raccoons, wood rats, dogs, and cats.
8. Housing conditions.
9. Blood transfusions, congenital infections and the use of contaminated hypodermic needles and syringes.
10. Facial swelling and a pronounced edema of the eyelids of one eye (Romaña sign).

11. An area of erythema and swelling develops called a chagoma.
12. Development of cardiac abnormalities.
13. 5 to 10%.
14. Damage to the muscles and nerves that control muscle tone of the hollow organs, particularly the heart, esophagus, and intestine.
15. The progressive loss of muscle tone of the esophagus and intestine resulting in decreased peristalsis and, eventually, a massive distension of the affected organs.
16. Wet mounts and stained blood smears.
17. U- or C-shaped bodies.
18. Because of the existence of other nonpathogenic trypanosomes that may be present.
19. Serum IgM levels are normally within limits in American trypanosomiasis.
20. Lysis centrifugation.

## 634

1. Promastigote.
2. In the Western Hemisphere, the sand flies are typically members of the genus *Lutzomyia*; in other parts of the world they usually belong to the genus *Phlebotomus*.
3. Within the phagocytic cells of the reticuloendothelial system and the endothelial cells of the capillaries.
4. They may remain localized in tissues near the site of the bite (cutaneous leishmaniasis; *L. tropica* and *L. mexicana*), may have a limited spread that involves more extensive areas of the skin and mucus membranes (mucocutaneous leishmaniasis; *L. braziliensis*), or may spread throughout the body and infect cells in many of the internal organs of the reticuloendothelial system, such as the spleen, liver, and bone marrow (visceral leishmaniasis; *L. donovani*).
5. When they feed on blood or host tissue juices containing cells infected with the amastigote stage of the parasite.
6. Cutaneous, mucocutaneous, and visceral leishmaniasis.
7. Visceral leishmaniasis (kala azar).
8. Moist ulcer and dry ulcer.
9. A cell-mediated, species specific, lifelong immunity against reinfection.
10. Secondary bacterial infections and diffuse cutaneous leishmaniasis.
11. It spreads to mucocutaneous borders and cartilaginous regions of the mouth and nose causing destruction and deformity of the nasal and oral passages.
12. They prefer to multiply in macrophages or other reticuloendothelial cells associated with the spleen, liver, bone marrow, and lymph nodes.
13. Fever and sweating at irregular intervals.
14. Demonstrating the intracellular amastigote in Giemsa-stained touch preparations of skin samples.
15. In vitro cultivation of the promastigote stages in Novy, MacNeal, Nicolle (NNN) medium.
16. Tissue from the spleen.

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## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter. When you have completed all unit review exercises, transfer your answers to ECI (AFIADL) Form 34, Field Scoring Answer Sheet.

**Do not return your answer sheet to AFIADL.**

59. (627) The exoerythrocytic stage of the malarial parasites takes place in
- bone.
  - the liver.
  - the intestine.
  - the erythrocytes.
60. (627) The **most** serious form of disease is caused by what malarial species?
- P. vivax*.
  - P. ovale*.
  - P. malariae*.
  - P. falciparum*.
61. (628) Which descriptions **best** describes the morphology of the macrogametocyte of *Plasmodium falciparum*?
- Cytoplasm generally darker blue.
  - Cytoplasm generally darker pink.
  - Diffused nuclear chromatin surrounding a dark-blue chromatin mass.
  - Slender and sausage-shaped, with a concentrated mass of dark pigment near the center.
62. (628) Which term **best** describes the appearance of merozoites of *Plasmodium malariae* in a single circle surrounding a large clump of hemozoin?
- Rosette.
  - Cartwheel.
  - Rosewood.
  - Cauliflower.
63. (629) The intraerythrocytic trophozoite stage of *Babesia* is almost identical to the ring form of
- P. ovale*.
  - P. vivax*.
  - P. malariae*.
  - P. falciparum*.
64. (630) In what cells of the cat does the first stage of the life cycle of *T. gondii* occur?
- White blood cells.
  - Parenchymal cells of the liver.
  - Epithelial cells of the small intestine.
  - Fixed macrophages of the lymph nodes.
65. (630) In **most** *T. gondii* infections, the patient
- remains asymptomatic.
  - develops lymphadenopathy.
  - develops inflammatory lesions of the organs infected.
  - experiences symptoms compatible with a diagnosis of mononucleosis or the flu.

66. (631) The two known stages of *P. carinii* are
- cyst and ova.
  - schizont and ova.
  - cyst and trophic form.
  - schizont and trophic form.
67. (631) It is assumed that **most** individuals contract pneumocystosis via
- blood transfusion.
  - inhalation of the cyst stage.
  - ingestion of food contaminated with the cyst stage.
  - the shared use of hypodermic needles and syringes.
68. (631) The **best** specimen for diagnosing pneumocystosis is
- feces.
  - sputum.
  - lymph node aspirates.
  - open lung biopsy material.
69. (632) How many days does it take for the development of the trypanosome infective stage within the tsetse fly?
- 7 to 14.
  - 15 to 23.
  - 25 to 50.
  - 60 to 120.
70. (632) The factor that limits the distribution of African trypanosomiasis is
- environmental conditions.
  - availability of susceptible hosts.
  - successful eradication programs.
  - the natural range of the insect vector.
71. (632) In the early stages of trypanosomiasis, what specimens can be used to detect trypomastigotes?
- Urine and sputum.
  - CSF and bone marrow.
  - Peritoneal fluid and liver aspirates.
  - Peripheral blood and lymph node aspirates.
72. (633) Of the cells the metacyclic trypomastigote enters when it invades the body, those especially penetrated are
- red blood cells (RBCs).
  - epithelial.
  - parenchymal.
  - fixed phagocytic.
73. (633) The **most** important factor in determining distribution of American trypanosomiasis is
- proper waste disposal.
  - geographic range of the insect vector.
  - the existence of a susceptible host population.
  - housing conditions of the human population at risk.



74. (634) What organism is responsible for New World cutaneous leishmaniasis?
- a. *L. tropica*.
  - b. *L. donovani*.
  - c. *L. mexicana*.
  - d. *L. aethiopica*.
75. (634) The **most** widely distributed form of leishmaniasis is
- a. visceral.
  - b. mucocutaneous.
  - c. old World cutaneous.
  - d. new World cutaneous.

When you complete this course, please complete the student survey on the Internet at this URL:  
[http://www.maxwell.af.mil/au/afiadl/operation/survey\\_fr.htm](http://www.maxwell.af.mil/au/afiadl/operation/survey_fr.htm).

## Student Notes

## Glossary of Terms

**Amastigote**—A “leishmania-form” organism that is the usual intracellular stage of *Leishmania* and *Trypanosoma* parasites.

**Biliary**—Pertaining to the bile, and bile ducts.

**Bronchoscopic**—Pertaining to bronchoscopy or to the bronchoscope and instrument for inspecting the interior of the bronchi

**Buccal**—Pertaining to or directed at the cheek.

**Cellulitis**—Inflammation of cellular tissue; especially purulent inflammation of the loose subcutaneous tissue.

**Cerebrospinal**—Pertaining to the brain and spinal cord.

**Cestode**—A common name applied to tapeworms as a group.

**Charcot-Leyden Crystals**—Perfectly formed, six-sided crystals, attenuated in the longitudinal axis, terminating in needlelike points at the poles.

**Chemotherapeutic**—Pertaining to chemotherapy.

**Chemotherapy**—The treatment of disease by chemical agents; first applied to use of chemicals that affect the causative organism unfavorably but do not harm the patient.

**Chromatography**—A method of chemical analysis in which the solution to be analyzed is poured into a vertical glass tube containing an adsorbent (or stationary phase), the different solutes moving through the stationary phase at different velocities according to their degree of attraction to it, and producing bands of color at different levels of the adsorption column.

**Cilia**—Minute hairlike structures which serve as organelles of locomotion in protozoans belonging to the class Ciliata.

**Ciliates**—A general term applied to protozoans of the class Ciliata, characterized by the presence of numerous fine hairlike fibrils on the surface of the body which serve as organelles of locomotion.

**Clot**—A semisolidified mass, as of blood or lymph; called also coagulum.

**Commensal**—Living on or within another organism, and deriving benefit without injuring or benefiting the other individual.

**Conjunctiva**—The delicate membrane that lines the eyelids and covers the exposed surface of the sclera.

**Cyst**—A structure, the outer covering of which consists of a protective layer which envelops the protoplasm of protozoans and enables them to survive under adverse environmental conditions.

**Cysticercosis**—The term applied to a disease in which the larval stage of a *Taenia* sp. invades body tissue.

**Cysticercus**—A larval form of *Taenia* sp. in which a single scolex is enclosed in a bladderlike cyst.

**Cytoplasmic**—Pertaining to or contained in cytoplasm.

**Diurnal**—Activity occurring during the day.

**Dysuria**—Painful or difficult urination.

**Echinococcosis**—The term applied to the disease which results from infection with the *Echinococcus* spp.

**Endocarditis**—Inflammation of the endocardium; a disease generally associated with rheumatic fever, and sometimes with other acute febrile diseases.

**Endocervical**—Pertaining to the interior of the cervix uteri.

**Endocervix**—The mucous membrane lining the canal of the cervix uteri.

**Endogenous**—Growing from within.

**Enterocolitis**—Inflammation involving both the small intestine and the colon.

**Etiologic**—Pertaining to the cause of a disease.

**Extracellular**—Outside a cell or cells.

**Extrapulmonary**—Not connected with the lungs.

**Fibrils**—Minute filaments which serve as organelles of locomotion in certain species of protozoans.

**Filariform**—The infective-stage larvae of hookworms and *Strongyloides*.

**Flagellate**—Any microorganism having flagella as organs of locomotion.

**Flagellum**—A mobile, whiplike process or stout cilium, especially a coiled filamentous appendage, originating in the cell wall or outer layers of cytoplasm of some rod-shaped bacteria, and serving as an organ of locomotion.

**Genitourinary**—Pertaining to the genital and urinary organs; urogenital; urinosexual.

**Helminth**—A general term applicable to the various species of worms which may be parasitic to man.

**Hemoflagellate**—Any flagellate protozoan parasite of the blood.

**Hemolysin**—A substance which liberates hemoglobin from red blood corpuscles by interrupting their structural integrity.

**Hemolysis**—The liberation of hemoglobin.

**Hemozoin**—The pigment found within malaria parasites. Also the pigment deposited in body tissue as a result of the rupture of infected red blood cells at the completion of the schizogenous cycle of the malaria parasite.

**Hepatotoxicity**—The quality or property of exerting a destructive or poisonous effect upon liver cells.

**Hexacanth**—The six-hooked embryo of certain species of tapeworms which is liberated from the egg at the time it hatches.

**Histoplasmosis**—Infection resulting from inhalation or, infrequently, the ingestion of spores of *Histoplasma capsulatum*.

**Homologous**—Corresponding in structure, position, origin, etc.

**Humoral**—Pertaining to the humors of the body.

**Hydatid**—The cyst stage of a tapeworm larva in which the cyst contains daughter cysts, each of which contains many scoleces.

**Immunodeficiency**—A deficiency in immune response, either in that mediated by humoral antibody or in that mediated by immune lymphoid cells.

**Immunoglobulins**—A protein of animal origin endowed with known antibody activity.

**Immunosuppression**—The artificial prevention or diminution of the immune response, as by irradiation or by administration of antimetabolites, antilymphocyte serum, or specific antibody.

**Intracellular**—Situated or occurring within a cell or cells.

**Lavage**—The irrigation or washing out of an organ, such as the stomach or bowel.

**Leshmaniasis**—Any of the three diseases caused by members of the genus *Leishmania*.

**Lymphoid**—Resembling or pertaining to lymph or tissue of the lymphatic system.

**Lymphoma**—A general term applied to any neoplastic disorder of the lymphoid tissue, including Hodgkin's disease.

**Meningeal**—Of or pertaining to the meninges.

**Meningitis**—Inflammation of the meninges.

**Meningoencephalitis**—Inflammation of the brain and meninges.

**Merozoite**—Asexual forms in the developmental cycle of the malaria parasite which are liberated into the blood stream when the schizont reaches maturity.

**Metabolite**—Any substance produced by metabolism or by a metabolic process.

**Metacercaria**—The encysted resting stage of a trematode either within the tissues of a crustacean or fish, or upon the surface of aquatic or semiaquatic vegetation.

**Microfilaria**—The prelarval stage of Filarioidea in the blood of man and tissues of the vector.

**Miracidium**—The free-swimming larva liberated into the water from the egg of a fluke at the time the egg hatches.

**Monoclonal**—Derived from a single cell.

**Mucosa**—A mucus membrane.

**Necrosis**—Death of tissue, usually as individual cells, groups of cells, or in small localized areas.

**Nematode**—A general term applicable to all species of roundworms.

**Oocyst**—The swollen saclike structure which develops in the stomach wall of the mosquito as a result of invasion by the zygote. When mature, it gives rise to the malarial sporozoites. Also, a stage in the development of coccidia.

**Operculum**—The cap which covers the opening through which embryo of certain species of flukes and tapeworms escape from the eggs at the time of hatching.

**Osteomyelitis**—Inflammation of bone caused by a pyogenic organism.

**Percutaneous**—Performed through the skin, as injection of radiopaque material in radiological examination, or the removal of tissue for biopsy accomplished with a needle.

**Pericardium**—The fibroserous sac that surrounds the heart and the roots of the great vessels.

**Phagocyte**—Any cell that ingests microorganisms or other cells and foreign particles.

**Pilus**—One of the filamentous appendages of the skin, consisting of modified epidermal tissue.

**Plasmid**—A generic term for all types of intracellular inclusions that can be considered as having genetic functions.

**Proglottids**—Individual divisions of the chain of segmentlike structures which make up the body of tapeworms, exclusive of the head and neck.

**Prostate**—A gland in the male which surrounds the neck of the bladder and the urethra.

**Rhabditiform**—Nematode larvae that have a muscular, bulbed esophagus.

**Schistosome**—The general term applied to the blood flukes.

**Schizogony**—The sexual cycle of sporozoa.

**Sporozoite**—The infective stage of the malarial parasite which migrates to the salivary gland of the mosquito.

**Subcutaneous**—Beneath the skin.

**Supernatant**—Situated above or on top of something.

**Trematode**—A common name applied to the flukes as a group.

**Trophozoite**—The active vegetative feeding motile stage of a protozoan.

**Trypanosome**—A blood and tissue flagellate of the genus, *Trypanosoma*.

**Umbilicate**—Shaped like or resembling the umbilicus.

**Umbonate**—Knoblike; button-like; having a button-like raised center.

**Urethritis**—Inflammation of the urethra.

**Vector**—A carrier, especially the animal (usually an arthropod) which transfers an infective agent from one host to another.

**Xenodiagnosis**—Diagnosis accomplished by allowing a known natural uninfected vector to feed upon a suspected infected individual for the purpose of attempting to recover the organism from the vector.

**Zoonotic**—A disease of animals that may be transmitted to humans under natural conditions.

## Acronyms

|                                   |                                       |
|-----------------------------------|---------------------------------------|
| <b>BF</b>                         | Bentonite flocculation                |
| <b>CF</b>                         | Complement fixation                   |
| <b>EDTA</b>                       | Ethylenediaminetetracetic acid        |
| <b>EIA</b>                        | Enzyme immunoassays                   |
| <b>ELISA</b>                      | Enzyme-linked ImmunoSorbent Assay     |
| <b>FOBT</b>                       | Fecal occult blood test               |
| <b>GI</b>                         | Gastrointestinal                      |
| <b>H<sub>2</sub>O<sub>2</sub></b> | Hydrogen peroxide                     |
| <b>IB</b>                         | Immunoblot                            |
| <b>IFA</b>                        | Indirect immunofluorescence test      |
| <b>IHA</b>                        | Hemaggultimation                      |
| <b>MIF</b>                        | Merthiolate-iodine formalin           |
| <b>NSAID</b>                      | Non-steroidal anti-inflammatory drugs |
| <b>OIF</b>                        | Operation Iraq Freedom                |
| <b>OLM</b>                        | Ocular larva migrans                  |
| <b>PVA</b>                        | Polyvinyl alcohol                     |
| <b>SAF</b>                        | Sodium acetate-acetic acid formalin   |
| <b>VLM</b>                        | Visceral larval migrans               |
| <b>WBC</b>                        | White blood cell                      |
| <b>WHO</b>                        | World Health Organization             |

## **Student Notes**



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## **Student Notes**

**CAREER DEVELOPMENT COURSE (CDC)**  
**4T051B, Medical Laboratory Journeyman, Volume 4**

**ASSESSMENT SURVEY**

**STUDENT NAME:**  
**(Optional)**

**DATE:**

**PURPOSE:** This survey is designed to obtain definitive and measurable feedback on the CDC volume you have just completed. It will provide us with your assessment of the quality of the training provided, identify areas where we may need to improve, and, with consolidation of the data, provide an overall assessment of how well we are doing in meeting your needs.

**INSTRUCTIONS:** Request you respond to the following statements. Please circle the appropriate response according to the following scale:

|                                                                                                                                | 1                    | 2        | 3                 | 4              | 5                 | 6     | 7                 |
|--------------------------------------------------------------------------------------------------------------------------------|----------------------|----------|-------------------|----------------|-------------------|-------|-------------------|
|                                                                                                                                | Strongly<br>Disagree | Disagree | Somewhat Disagree | Not Applicable | Somewhat<br>Agree | Agree | Strongly<br>Agree |
| 1. The information presented in the CDC provided me with knowledge required to perform on the job.                             | 1                    | 2        | 3                 | 4              | 5                 | 6     | 7                 |
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| a. well written,                                                                                                               | 1                    | 2        | 3                 | 4              | 5                 | 6     | 7                 |
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| d. the information was easily understood.                                                                                      | 1                    | 2        | 3                 | 4              | 5                 | 6     | 7                 |
| 4. The provided figures consistently supported the text illustrating and/or clarifying principles, techniques, and procedures. | 1                    | 2        | 3                 | 4              | 5                 | 6     | 7                 |
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| 6. Self-test Questions and Unit Review Exercises:                                                                              |                      |          |                   |                |                   |       |                   |
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