Microbiology in college courses is usually dominated by bacteriology, but the discipline also includes eukaryotic microscopic organisms. You have had the opportunity to look at some eukaryotic microorganisms in Exercise 3-3. In this section, you will examine some representative protozoans and some microscopic fungi that are of medical or commercial importance. These exercises are followed by one dealing with parasitic helminths (worms) because their identification is often the responsibility of microbiologists who examine patient samples. Thus, these parasites have entered the domain of microbiology.
Exercise 11-1

The Fungi—Common Yeasts and Molds

Members of the Kingdom Fungi are nonmotile eukaryotes. Their cell wall is usually made of the polysaccharide chitin, not cellulose as in plants. Unlike animals (that ingest, then digest their food), fungi are absorptive heterotrophs; they secrete exoenzymes into the environment, then absorb the digested nutrients. Most are saprophytes that decompose dead organic matter, but some are parasites of plants, animals, or humans.

Fungi are informally divided into unicellular yeasts (Figure 11-1) and filamentous molds (Figure 11-2) based on their overall appearance. Dimorphic fungi have both mold and yeast life-cycle stages. Filamentous fungi that produce fleshy reproductive structures—mushrooms, puffballs, and shelf fungi—are referred to as macrofungi (Figure 11-3), even though the majority of the fungus is filamentous and hidden underground or within decaying matter.

Individual fungal filaments are called hyphae, and collectively they form a mycelium. The hyphae are darkly pigmented in dematiaceous fungi and unpigmented in hyaline or moniliaceous fungi (Figure 11-4). Hyphae may be septate, in which walls separate adjacent cells, or nonseptate if walls are absent (Figure 11-5).

Fungal life cycles usually are complex, involving both sexual and asexual forms of reproduction. Gametes are produced by gametangia, and spores are produced by a variety of sporangia. Typically, the only diploid cell in the fungal life cycle is the zygote, which undergoes meiosis to produce haploid spores that are characteristic of the fungal group (see the following paragraph). Various asexual spores also may be produced during the life cycle of many fungi. If they form at the ends of hyphae, they are called conidia. Other asexual spores are blastospores, which are produced by budding, and arthrospores, which are produced when a hypha breaks.

Chlamydospores (chlamydoconidia) are formed at the end of some hyphae and are a resting stage.

Formal taxonomic categories are based primarily on the pattern of sexual spore production and the presence of crosswalls in the hyphae. Members of the Class Zygomycetes are terrestrial, have nonseptate hyphae, and produce nonmotile sporangiospores and zygospores. Members of the Class Ascomycetes produce a sac (an ascus) in which the zygote undergoes meiosis to produce haploid ascospores. Ascomycete hyphae are septate. Members of the Class Basidiomycetes have septate hyphae and during sexual reproduction produce a basidium that undergoes meiosis to produce four basidiospores attached to its surface. The Class Deuteromycetes is an unnatural assemblage of fungi in which sexual stages are either unknown or are not used in classification. Most deuteromycetes resemble ascomycetes.

Following is a brief survey of fungi that are likely to be encountered in an introductory microbiology class and selected medically important fungi.

**FIGURE 11-1 Yeast**
This is a wet mount of the Brewer's yeast, *Saccharomyces cerevisiae* stained with Methylene Blue. (X600)

**FIGURE 11-2 Mold**
Molds grow as fuzzy colonies. Their spores are abundant in the environment and frequently show up as contaminants on agar plates.
Yeasts of Medical or Economic Importance

*Candida albicans*

*Candida albicans* (Figure 11-6) is part of the normal respiratory, gastrointestinal and female urogenital tract flora. Under the proper circumstances, it may flourish and produce pathological conditions, such as thrush in the oral cavity, vulvovaginitis of the female genitals, and cutaneous candidiasis of the skin. Systemic candidiasis may follow infection of the lungs, bronchi, or kidneys. Entry into the blood may result in endocarditis. Individuals most susceptible to *Candida* infections are diabetics, those with immunodeficiency (e.g., AIDS), catheterized
patients, and individuals taking antimicrobial medications. Budding results in chains of cells called pseudohyphae, which produce clusters of round, asexual blastoconidia at the cell junctions. Large, round, thick-walled chlamydospores form at the ends of pseudohyphae.

**Saccharomyces cerevisiae**

*Saccharomyces cerevisiae* is an ascomycete used in the production of bread, wine, and beer but is not an important human pathogen. It does not form a mycelium but, rather, produces a colony similar to bacteria (Figure 11-7). The vegetative cells (blastoconidia) are generally oval to round in shape, and asexual reproduction occurs by budding (Figure 11-8). Short pseudohyphae are sometimes produced when the budding cells fail to separate. Meiosis produces one to four ascospores within the vegetative cell, which acts as the ascus. Ascospores may fuse to form another generation of diploid vegetative cells or they may be released to produce a population of haploid cells that are indistinguishable from diploid cells. Haploid cells of opposite mating types may also combine to create a diploid cell.

**Molds of Medical or Economic Importance**

**Rhizopus**

*Rhizopus* species are fast-growing zygomycetes that produce white or grayish, cottony growth. The mycelium becomes darker with age as sporangia are produced, giving it a “salt and pepper” appearance (Figure 11-9). Microscopically, *Rhizopus* species produce broad (10 μm), hyaline, and usually nonseptate surface and aerial hyphae.ANCHORING rhizoids (Figure 11-10) are produced where the surface hyphae (stolons) join the bases of the long, unbranched sporangiophores.

The *Rhizopus* life cycle (Figure 11-11) has both sexual and asexual phases. Asexual sporangiospores are produced by large, circular sporangia (Figure 11-12) borne at the ends of long, nonseptate, elevated sporangiophores. A hemispherical *columella* supports the sporangium. The spores develop into hyphae that are identical to those that produced them. On occasion, sexual

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**FIGURE 11-7** *Saccharomyces cerevisiae* Colony

Note that the appearance is similar to a typical bacterial colony, not “fuzzy” like mold colonies.

**FIGURE 11-8** Wet Mount of *Saccharomyces cerevisiae*

Vegetative Cells (Crystal Violet Stain, X1320)

Note the budding cell (blastoconidium) in the center of field (arrow). This wet mount was stained with Methylene Blue.

**FIGURE 11-9** *Rhizopus stolonifer*

Black asexual sporangia of this bread mold have begun to form, giving the growth a “salt and pepper” appearance.
reproduction occurs when hyphae of different mating types (designated + and − strains) make contact.

Initially, progametangia (Figure 11-13) extend from each hypha. Upon contact, a septum separates the end of each progametangium into a gamete (Figure 11-14). The walls between the two gametangia dissolve, and a thick-walled zygospore develops (Figures 11-15 and 11-16). Fusion of nuclei occurs within the zygospore and produces one or more diploid nuclei, or zygotes. After a dormant period, meiosis of the zygotes occurs. The zygospore then germinates and produces a sporangium similar to asexual sporangia. Haploid spores are released, develop into new hyphae, and the life cycle is completed.

*Rhizopus* species are common contaminants. *R. stolonifer* is the common bread mold. *R. oryzae* and *R. arrhizus* are responsible for producing zygomycosis, a condition found most frequently in diabetics and immunocompromised patients. Inhalation of spores may lead to hypersensitivity reactions in the respiratory system. Entry into the blood leads to rapid spreading of the organism, occlusion of blood vessels, and necrosis of tissues.

**FIGURE 11-10 RHIZOPUS RHIZOIDS (X200)**
Anchoring rhizoids form at the junction of each sporangio- phore (SP) and the stolon (ST). Note the absence of the septa.
**Aspergillus**

The genus *Aspergillus* is characterized by green to yellow or brown granular colonies with a white edge (Figure 11-17). One species, *A. niger*, produces distinctive black colonies. Vegetative hyphae are hyaline (unpigmented) and septate. The *Aspergillus* fruiting body is distinctive, with chains of conidia arising from one (uniseriate) or two (biseriate) rows of phialides attached to a swollen vesicle at the end of an unbranched conidiophore (Figure 11-18). The conidiophore grows from a foot cell in the vegetative hypha (Figure 11-19). Fruiting body structure and size, and conidia color are useful in species identification.

*A. fumigatus* and other species are opportunistic pathogens that cause aspergillosis, an umbrella term covering many diseases. One form of pulmonary aspergillosis (referred to as fungus ball) involves colonization of the bronchial tree or tissues damaged by tuberculosis.
Allergic aspergillosis may occur in individuals who are in frequent contact with the spores and become sensitized to them. Subsequent contact produces symptoms similar to asthma. Invasive aspergillosis is the most severe form. It results in necrotizing pneumonia and may spread to other organs.

Some species of *Aspergillus* are of commercial importance. Fermentation of soybeans by *A. oryzae* produces soy paste. Soy sauce is produced by fermenting soybeans with a mixture of *A. oryzae* and *A. sojae*. *Aspergillus* is also used in commercial production of citric acid.

**Penicillium**

Members of the genus *Penicillium* produce distinctive green, powdery, radially furrowed colonies with a white apron (Figure 11-20) and light colored reverse surface. The hyphae are septate and thin. Distinctive *Penicillium* fruiting bodies, consisting of *metulae, phialides* and chains of spherical conidia, are located at the ends of branched or unbranched *conidiophores* (Figure 11-21). Although not an important feature in laboratory identification, sexual reproduction results in the formation of ascospores within an ascus.

*Penicillium* is best known for its production of the antibiotic penicillin, but it is also a common contaminant. One pathogen, *P. marneffei*, is endemic to Asia and is responsible for disseminated opportunistic infections of the lungs, liver, and skin in immunosuppressed and immunocompromised patients. It is thermally dimorphic, producing a typical velvety colony with a distinctive red pigment at 25°C but converting to a yeast form at 35°C. Other species of *Penicillium* are of commercial importance for fermentations used in cheese production. Examples include *P. roquefortii* (Roquefort cheese) and *P. camemberti* (Camembert and Brie cheeses).
Materials

Per Student Group

- Agar slant of Saccharomyces cerevisiae
- Potato Dextrose Agar or Sabouraud Dextrose Agar plate culture of Aspergillus spp. (with lid taped on)
- Potato Dextrose Agar or Sabouraud Dextrose Agar plate culture of Penicillium spp. (with lid taped on)
- Potato Dextrose Agar or Sabouraud Dextrose Agar plate culture of Rhizopus spp. (with lid taped on)
- Gram’s iodine stain or Methylene Blue
- Dissecting microscope
- Prepared slides of:
  - Aspergillus spp. conidiophore
  - Candida albicans
  - Penicillium spp. conidiophore
  - Rhizopus spp. sporangia
  - Rhizopus spp. gametangia

Procedure

Yeast

1. Using an inoculating loop and aseptic technique, make a wet mount slide of Saccharomyces cerevisiae as illustrated in Figure 3-18, and stain with iodine or Methylene Blue. Observe under high dry and oil immersion. Identify vegetative cells and budding cells. Sketch representative cells in the space provided on the Data Sheet.

2. Observe prepared slides of Candida albicans. Identify vegetative cells and budding cells. Sketch representative cells in the space provided on the Data Sheet.

Molds

1. Obtain the plate culture of Rhizopus. Do not remove the lid. Uncovering the organism will spread spores and contaminate the laboratory.
   a. Examine the colony morphology, and sketch a representative colony in the space provided on the Data Sheet. Record the color on both the front (obverse) and reverse surfaces. Also record the colony texture as glabrous (leathery), velvety, yeast-like, cottony, or granular (powdery), and the colony topography as flat, rugose (with radial grooves), folded, crateriform, verrucose (warty, rough) or cerebriform (brain-like).
   b. Examine the colony under the dissecting microscope and identify hyphae, rhizoids, and sporangia. Sketch and label representative structures in the space provided on the Data Sheet.

2. Examine prepared slides of Rhizopus sporangia using medium and high dry powers. Identify the following: sporangiophores, sporangia, and spores. Sketch and label representative structures in the space provided on the Data Sheet.

3. Examine prepared slides of Rhizopus gametangia using medium and high dry power. Identify the following: progametangia, gametangia, young zygosporangia, mature zygosporangia. Sketch and label representative structures in the space provided on the Data Sheet.
4. Obtain the plate culture of *Penicillium*. Do not remove the lid from the plate or you will spread spores and contaminate the laboratory.
   a. Examine the colony morphology and sketch a representative colony in the space provided on the Data Sheet. Record the color on both the front (obverse) and reverse surfaces. Also record the colony texture as glabrous (leathery), velvety, yeast-like, cottony, or granular (powdery), and the colony topography as flat, rugose (with radial grooves), folded, crateriform, verrucose (warty, rough) or cerebriform (brain-like).
   b. Examine the colony under the dissecting microscope. Identify hyphae and conidia. Sketch and label representative structures in the space provided on the Data Sheet.

5. Observe prepared slides of *Penicillium* conidiophores. Identify the following: hyphae, conidiophores, and chains of conidia. Sketch and label representative structures in the space provided on the Data Sheet.

6. Obtain the plate culture of *Aspergillus*. Do not remove the lid from the plate or you will spread spores and contaminate the laboratory.
   a. Examine the colony morphology and sketch a representative colony in the space provided on the Data Sheet. Record the color on both the front (obverse) and reverse surfaces. Also record the colony texture as glabrous (leathery), velvety, yeast-like, cottony, or granular (powdery), and the colony topography as flat, rugose (with radial grooves), folded, crateriform, verrucose (warty, rough) or cerebriform (brain-like).
   b. Examine the colony under the dissecting microscope. Identify hyphae and conidia. Sketch and label representative structures in the space provided on the Data Sheet.

7. Observe prepared slides of *Aspergillus* conidiophores. Identify hyphae, conidiophores, and conidia. Sketch and label representative structures in the space provided on the Data Sheet.

References
Exercise 11-2
Examination of Common Protozoans of Clinical Importance

Protozoans are unicellular eukaryotic heterotrophic microorganisms. A typical life cycle includes a vegetative trophozoite stage and a resting cyst stage. Some have additional stages, making their life cycles more complex.

As discussed in Exercise 3-3, protozoans are classified as follows:

Phylum Sarcomastigophora (including Subphylum Mastigophora [the flagellates] and Subphylum Sarcodina [the amoebas]),
Phylum Ciliophora (the ciliates), and
Phylum Apicomplexa (sporozoans and others).

Following is a survey of some commonly encountered protozoans of clinical importance. You will be examining these on prepared slides because most are pathogens that are not handled appropriately in a beginning microbiology laboratory.

Amoeboid Protozoans Found in Clinical Specimens

*Entamoeba histolytica*

*Entamoeba histolytica* is the causative agent of amoebic dysentery (amebiasis), a disease most common in areas with poor sanitation. Identification is made by finding either trophozoites (Figure 11-22) or cysts (Figure 11-23) in a stool sample. The diagnostic features of each are described in the captions.

Infection occurs when a human host ingests cysts, either through fecal–oral contact or, more typically, contaminated food or water. Cysts (but not trophozoites) are able to withstand the acidic environment of the stomach. Upon entering the less acidic small intestine, the cysts undergo excystation. Mitosis produces eight small trophozoites from each cyst.

The trophozoites parasitize the mucosa and submucosa of the colon, causing ulcerations. They feed on red blood cells and bacteria. The extent of damage determines whether the disease is acute, chronic, or asymptomatic. In the most severe cases, infection may extend to other organs, especially the liver, lungs, or brain. Among the symptoms of amoebic dysentery are abdominal pain, diarrhea, blood and mucus in feces, nausea, vomiting, and hepatitis.

![Figure 11-22](image-url) *Entamoeba histolytica* Trophozoite (X800, Iron Hematoxylin Stain)

Trophozoites range in size from 12 to 60 μm. Notice the small, central karyosome, the beaded chromatin at the margin of the nucleus, the ingested red blood cells, and the finely granular cytoplasm. Compare with an *Entamoeba coli* trophozoite in Figure 11-24.

![Figure 11-23](image-url) *Entamoeba histolytica* Cysts (A) Cysts are spherical with a diameter of 10 to 20 μm. Two of the four nuclei are visible; other nuclear characteristics are as in the trophozoite. Compare with an *Entamoeba coli* cyst in Figure 11-25 (X1320, Iron Hematoxylin Stain). (B) *E. histolytica* cyst (X1200, Trichrome Stain) with cytoplasmic chromatoidal bars (CB). These are found in approximately 10% of the cysts, have blunt ends, and are composed of ribonucleoprotein.
Developing cysts undergo mitosis to produce mature quadrinucleate cysts, which are shed in the feces and are infective. They also may persist in the original host, resulting in an asymptomatic carrier—a major source of contamination and infection.

Another member of the genus *Entamoeba* deserves mention here. *Entamoeba coli* is a fairly common, non-pathogenic intestinal commensal that must be differentiated from *E. histolytica* in stool samples. Its characteristic features are given in the captions to Figures 11-24 and 11-25.

**Ciliate Protozoan Found in Clinical Specimens**

*Balantidium coli*

*Balantidium coli* (Figures 11-26 and 11-27) is the causative agent of balantidiasis and exists in two forms: a vegetative trophozoite and a cyst. Laboratory diagnosis is made by identifying either the cyst or the trophozoite, with the latter being more commonly found.

The trophozoite is highly motile because of the cilia and has a macronucleus and a micronucleus. Cysts in sewage-contaminated water are the infective form. Trophozoites may cause ulcerations of the colon mucosa, but not to the extent produced by *Entamoeba histolytica*. Symptoms of acute infection are bloody and mucoid feces. Diarrhea alternating with constipation may occur in chronic infections. Most infections probably are asymptomatic.
Flagellate Protozoans Found in Clinical Specimens

Giardia lamblia
Giardiasis is caused by *Giardia lamblia* (also known as *Giardia intestinalis*), a flagellate protozoan. It is seen most frequently in the duodenum as a heart-shaped vegetative trophozoite (Figure 11-28) with four pairs of flagella and a sucking disc that allows it to resist gut peristalsis. Multinucleate cysts lacking flagella (Figure 11-29) are formed as the organism passes through the colon. Cysts are shed in the feces and may produce infection of a new host upon ingestion. Transmission typically involves fecally contaminated water or food, but direct fecal-oral contact transmission is also possible.

The organism attaches to epithelial cells but does not penetrate to deeper tissues. Most infections are asymptomatic. Chronic diarrhea, dehydration, abdominal pain, and other symptoms may occur if the infection produces a population large enough to involve a significant surface area of the small intestine. Diagnosis is made by identifying trophozoites or cysts in stool specimens.

Trichomonas vaginalis
*Trichomonas vaginalis* (Figure 11-30) is the causative agent of trichomoniasis (vulvovaginitis) in humans. It has four anterior flagella and an undulating membrane.

Trichomoniasis may affect both sexes but is more common in females. *T. vaginalis* causes inflammation

**Figure 11-27** *Balantidium coli* Cyst (X1000)
Cysts usually are spherical and have a diameter in the range of 50 to 75 μm. There is a cyst wall, and the cilia are absent. As in the trophozoite, the macronucleus is prominent, but the micronucleus may not be.

**Figure 11-28** *Giardia lamblia* Trophozoite (X1320, Iron Hematoxylin Stain)
Trophozoites have a long, tapering posterior end and range in size from 9 to 21 μm by 5 to 15 μm. There are two nuclei with small karyosomes. Two median bodies are visible, but the four pairs of flagella are not.

**Figure 11-29** *Giardia lamblia* Cysts (X1000, Trichrome Stain).
Giardia cysts are smaller than trophozoites (8 to 12 μm by 7 to 10 μm), but the four nuclei with eccentric karyosomes and the median bodies (M) are still visible.

**Figure 11-30** *Trichomonas vaginalis* (X2027)
The trophozoite is the only stage of the *Trichomonas* life cycle. Several flagella are visible.
of genitourinary mucosal surfaces—typically the vagina, vulva, and cervix in females and the urethra, prostate, and seminal vesicles in males. Most infections are asymptomatic or mild. Some erosion of surface tissues and a discharge may be associated with infection. The degree of infection is affected by host factors, especially the bacterial flora present and the pH of the mucosal surfaces. Transmission typically is by sexual intercourse.

The morphologically similar nonpathogenic *Trichomonas tenax* and *T. hominis* are residents of the oral cavity and intestines, respectively.

**Trypanosoma brucei**

*Trypanosoma brucei* (Figure 11-31) is a species of flagellated protozoans divided into subspecies: *T. brucei brucei* (which is nonpathogenic), and *T. brucei gambiensense* and *T. brucei rhodesiense*, which produce African trypanosomiasis, also known as African sleeping sickness. The organisms are very similar morphologically but differ in geographic range and disease progress. West African trypanosomiasis (caused by *T. brucei gambiensense*) is generally a mild, chronic disease that may last for years, whereas East African trypanosomiasis (caused by *T. brucei rhodesiense*) is more acute and results in death within a year. Modern molecular methods that compare proteins, RNA, and DNA are used to differentiate between them.

Trypanosomes have a complex life cycle. One stage of the life cycle, the epimastigote, multiplies in an intermediate host, the tsetse fly (genus *Glossina*).

The infective trypomastigote stage then is transmitted to the human host through tsetse fly bites. Once introduced, trypomastigotes multiply and produce a chancre at the site of the bite. They enter the lymphatic system and spread through the blood, and ultimately to the heart and brain.

Immune response to the pathogen is hampered by the trypanosome’s ability to change surface antigens faster than the immune system can produce appropriate antibodies. This antigenic variation also makes development of a vaccine unlikely. Diagnosis is made from clinical symptoms and identification of the trypomastigote in patient specimens (e.g., blood, CSF, and chancre aspirate). An ELISA and an indirect agglutination test also have been developed to detect trypanosome antigens in patient samples.

Progressive symptoms include headache, fever, and anemia, followed by symptoms characteristic of the infected sites. The symptoms of sleeping sickness—sleepiness, emaciation, and unconsciousness—begin when the central nervous system becomes infected. Depending on the infecting strain, the disease may last for months or years, but the mortality rate is high. Death results from heart failure, meningitis, or severe debility of some other organ(s).

The infective cycle is complete when an infected individual (humans, cattle, and some wild animals are reservoirs) is bitten by a tsetse fly, which ingests the organism during its blood meal. It becomes infective for its lifespan.

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**Sporozoan Protozoans Found in Clinical Specimens**

**Plasmodium spp.**

Plasmodia are sporozoan parasites with a complex life cycle, part of which is in various vertebrate tissues while the other part involves an insect. In humans, the tissues are the liver and red blood cells, and the insect vector is the female *Anopheles* mosquito. A generalized life cycle is shown in Figure 11-32. Representative life cycle stages for the various species are shown in Figures 11-33 to 11-35.

Four species of *Plasmodium* cause malaria in humans:

- *P. vivax* (benign tertian malaria),
- *P. malariae* (quartan malaria),
- *P. falciparum* (malignant tertian malaria), and
- *P. ovale* (ovale malaria).

The life cycles are similar for each species, as is the progress of the disease, so *P. falciparum* will be discussed as an example, with unique aspects compared
**FIGURE 11-32** *Plasmodium* Life Cycle

**FIGURE 11-33** *Plasmodium falciparum* Double Infection of a Red Blood Cell (X2640)
Double infections are commonly seen in *P. falciparum* infections. A single infection is seen at the right. Young trophozoites are said to be in the “ring stage.”

**FIGURE 11-34** A Mature *Plasmodium vivax* Schizont Composed of Approximately 16 Merozoites (X1200)
More than 12 merozoites distinguish *P. vivax* from *P. malariae* and *P. ovale*, both of which typically have eight, but up to 12 merozoites. *P. falciparum* may have up to 24 merozoites, but they typically are not seen in peripheral blood smears and so are not confused with *P. vivax*. 
projections that cause them to adhere to the lining of small blood vessels. This can lead to obstruction of the vessels, thrombosis, or local ischemia, which account for many of the fatal complications of this type of malaria—including liver, kidney, and brain damage.

**Toxoplasma gondii**

Like other sporozoans, the *Toxoplasma gondii* (Figure 11-36) life cycle has sexual and asexual phases. The sexual phase occurs in the lining of cat intestines where oocysts are produced and shed in the feces. Each oocyst undergoes division and contains eight sporozoites. If ingested by another cat, the sexual cycle may be repeated as the sporozoites produce gametocytes, which in turn produce gametes. If ingested by another animal host (including humans) the oocyst germinates in the duodenum and releases the sporozoites. Sporozoites enter the blood and infect other tissues, where they become trophozoites, which continue to divide and spread the infection to lymph nodes and other parts of the reticuloendothelial system. Trophozoites ingested by a cat eating an infected animal develop into gametocytes in the cat's intestines. Gametes are formed, fertilization produces an oocyst, and the life cycle is completed.

Infection via ingestion of the oocyst typically is not serious. The infected person may notice fatigue or muscle aches. The more serious form of the disease involves infection of a fetus across the placenta from an infected mother. This type of infection may result in stillbirth, or liver damage and brain damage. AIDS patients may incur fatal complications from infection.

**Materials**

**Per Student Group**

- Prepared slides of:
  - *Entamoeba histolytica* trophozoite and cyst
  - *Entamoeba coli* trophozoite and cyst
  - *Balantidium coli* trophozoite and cyst

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**FIGURE 11-35 Plasmodium falciparum Gametocyte in an Erythrocyte (X1000)**

Differentiation between microgametocytes and megagametocytes is difficult in this species. The erythrocyte membrane is visible around the gametocyte (arrow).

**FIGURE 11-36 Toxoplasma gondii Trophozoites (X1000)**

Notice the bow-shaped cells and the prominent nuclei.
Giardia lamblia trophozoite and cyst
• Trichomonas vaginalis trophozoite
• Trypanosoma spp.
• Plasmodium spp.
• Toxoplasma gondii trophozoite

Procedure
1. Obtain prepared slides of the protozoan pathogens and observe them under appropriate magnification. You should observe the assigned structures on each organism. (Many of these slides are made from patient samples, so there will be a lot of other material on the slide besides the desired organism. You must search carefully and with patience.)
2. Sketch and label the assigned components on the Data Sheet.

Entamoeba histolytica
• Trophozoite
  • pseudopods
  • nucleus with small, central karyosome and beaded nucleus
  • ingested erythrocytes
• Cyst
  • multiple nuclei (up to four) with karyosomes and chromatin as in the trophozoite
  • cytoplasmic chromatoidal bars (maybe)

Entamoeba coli
• Trophozoite
  • same as E. histolytica except with eccentric karyosome and unclumped chromatin
• Cyst
  • up to eight nuclei (more than four is enough to distinguish it from E. histolytica) that are the same as in the trophozoite
  • cytoplasmic chromatoidal bars (maybe)

Balantidium coli
• Trophozoite
  • elongated shape
  • cilia
  • macronucleus
  • micronucleus (maybe)
• Cyst
  • spherical shape with multiple nuclei

Giardia lamblia
• Trophozoite
  • oval shape
  • flagella (four pairs)
  • nuclei (two)
  • median bodies (two)
• Cyst
  • multiple nuclei (four)
  • median bodies (four)

Trichomonas vaginalis trophozoite
• nucleus
• flagella (four)

Trypanosoma spp.
• nucleus
• flagellum
• undulating membrane
• kinetoplast

Plasmodium spp.
• ring stage
• mature trophozoite
• schizont
• male gametocyte
• female gametocyte

Toxoplasma gondii
• trophozoite
• bow-shaped cells
• nucleus

References
Exercise 11-3
Parasitic Helminths

A study of helminths is appropriate to the microbiology lab because clinical specimens may contain microscopic evidence of helminth infection. The three major groups of parasitic worms encountered in lab situations are trematodes (flukes), cestodes (tapeworms), and nematodes (round worms). Life cycles of the parasitic worms are often complex, sometimes involving several hosts, and are beyond the scope of this book. Emphasis here is on a brief background and clinically important diagnostic features of selected worms.

Trematode Parasites Found in Clinical Specimens

Clonorchis (Opisthorchis) sinensis

Clonorchis sinensis is the Oriental liver fluke (Figure 11-37) and causes clonorchiasis, a liver disease. It is a common parasite of people living in Japan, Korea, Vietnam, China, and Taiwan and is becoming more common in the United States with the influx of Southeast Asian immigrants. Infection typically occurs when undercooked infected fish is ingested. The adults migrate to the liver bile ducts and begin laying eggs in approximately one month. Degree of damage to the bile duct epithelium and surrounding liver tissue is due to the number of worms and the duration of infection. Diagnosis is made by identifying the characteristic eggs in feces (Figure 11-38).

Paragonimus westermani

Paragonimus westermani (Figure 11-39) is a lung fluke and one of several species to cause paragonimiasis, a disease mostly found in Asia, Africa, and South America. P. westermani is primarily a parasite of carnivores, but humans (omnivores) may get infected when eating undercooked crabs or crayfish infected with the cysts. Ingested juveniles excyst in the duodenum and travel to the abdominal wall. After several days, they resume their journey and find their way to the bronchioles where the adults mature. Eggs (Figure 11-40) are released in approximately two to three months and are diagnostic of infection. They may be recovered in sputum, lung fluids, or feces. Consequences of lung infection are a local inflammatory response followed by possible ulceration. Symptoms include cough with discolored or bloody sputum and difficulty breathing. These cases are rarely fatal, but may last a couple of decades. Occasionally, the wandering juveniles end up in other tissues, such as the brain or spinal cord, which can cause paralysis or death.
**Paragonimus westermani Egg**

Paragonimus westermani eggs are ovoid and range in size from 80 to 120 μm long by 45 to 70 μm wide. They have an operculum (O) and the shell is especially thick at the abopercular end (arrow). They are unembryonated when seen in feces.

**Schistosoma mansoni**

Schistosoma mansoni (Figure 11-41) is found in Brazil, some Caribbean islands, Africa, and parts of the Middle East. Infection occurs via contact with fecally contaminated water containing juveniles of the species. The juveniles penetrate the skin, enter circulation and continue development in the intestinal veins. Eggs (Figure 11-42) from the adults penetrate the intestinal wall and are passed out with the feces. Presence of eggs in the feces indicates infection. Some patients are asymptomatic, whereas others have bloody diarrhea, abdominal pain, and lethargy.

**Cestode Parasites Found in Clinical Specimens**

**Dipylidium caninum**

Dipylidium caninum (Figure 11-43) is a common parasite of dogs and cats. Human infection usually occurs in children. The adult worms reside in the dog or cat intestines and release proglottids (Figure 11-44) containing egg packets that migrate out of the anus. When these dry, they look like rice grains. Larval fleas may eat the eggs and become infected. If the dog, cat, or child ingests one of these fleas, the life cycle is completed in the new host. Infection may be asymptomatic or produce mild
abdominal discomfort, loss of appetite and indigestion. Diagnosis is made by identifying the egg packets (Figure 11-45).

**Echinococcus granulosus**

The definitive host of *Echinococcus granulosus* (Figure 11-46) is a carnivore, but the life cycle requires an intermediate host, usually an herbivorous mammal. Humans involved in raising domesticated herbivores (e.g., sheep with their associated dogs) are most susceptible as intermediate hosts and develop hydatid disease. Ingestion of a juvenile *E. granulosus* leads to development of a hydatid cyst in the lung, liver, or other organ, a process that may take many years. The cyst (Figure 11-47) has a thick wall and develops many protoscolices within (Figure 11-48). The protoscolices, if ingested, are infective to the definitive host. Symptoms depend on the location and size of the hydatid cyst, which interferes with normal organ function. Due to sensitization by the parasite’s antigens, release of fluid from the cyst can result in anaphylactic shock of the host. Diagnosis is made by detection of the cyst by ultrasound or X-ray.

**FIGURE 11-44 DIPYLDIUM CANINUM PROGLOTTIDS (X40)**
Visible are the testes (T), vasa deferentia (VD), ovaries (O), and vitelline glands (VG). The reproductive openings (arrows) on each side of the proglottid give this worm its common name—the “double-pored tapeworm.”

**FIGURE 11-45 DIPYLDIUM CANINUM EGG PACKET (X1000)**
Each *Dipylidium caninum* egg packet is composed of 5 to 15 eggs each with an onchosphere (O). The onchosphere contains six hooklets.

**FIGURE 11-46 ECHINOCOCUS GRANULOSUS ADULT (X20)**
Adult worms are about 0.5 cm in length. There is a scolex with a ring of hooks, a neck, and one proglottid that contains up to 1500 eggs.

**FIGURE 11-47 ECHINOCOCUS GRANULOSUS CYST IN LUNG TISSUE (SEC., X96)**
The cyst wall consists of a fibrous layer of host tissue (F), an acellular layer (A), and a germinal epithelium (G) that gives rise to stalked brood capsules (B). Brood capsules produce many *E. granulosus* protoscolices (P).
**FIGURE 11-48 Echinococcus granulosus Protoscolex**
(L.S., X320)
The protoscolex contains an invaginated scolex with hooks (H). Upon ingestion by the host, the protoscolex evaginates and produces an infectious scolex that attaches to the intestinal wall, matures and produces eggs.

**Hymenolepis (Vampirolepis) nana**

*Hymenolepis nana* (Figures 11-49 and 11-50) is the dwarf tapeworm and is the most common cestode parasite of humans in the world. When eggs (Figure 11-51) are ingested, the oncospheres develop into juveniles in the lymphatics of intestinal villi. These juveniles are then released into the lumen within a week and attach to the mucosa to mature into adults. Infection may involve hundreds of worms, yet symptoms are usually mild: diarrhea, nausea, loss of appetite, or abdominal pain. Eggs may reinfest the same host or pass out with the feces to infect a new host. Eggs in the feces are used for identification, but proglottids are not as they are rarely passed.

**Taenia spp.**

Two taenid worms are important human pathogens. These are *Taenia saginata* (*Taeniarhynchus saginatus*) —the beef tapeworm—and *Taenia solium*—the pork tapeworm.

*T. saginata* infects humans who eat undercooked beef containing juvenile worms. In the presence of bile salts, the juveniles develop into adults and begin producing gravid proglottids within a few weeks. Symptoms of infection are usually mild nausea, diarrhea, abdominal pain, and headache. Diagnosis to species is impossible with only the eggs (Figure 11-52); specific identification requires a scolex or gravid proglottid (Figure 11-53).

**FIGURE 11-49 Hymenolepis nana Scolex (X40)**
Adults gain a length of up to 10 cm, but are only 1 mm in width. The rostellum (R) is armed with up to 30 hooks (H).

**FIGURE 11-50 Hymenolepis nana Proglottids (X40)**
*H. nana* is hermaphroditic, but in this specimen, the numerous testes obscure most other structures.

**FIGURE 11-51 Hymenolepis nana Egg in Feces**
(X1000, D'Antoni's Iodine Stain)
The *Hymenolepis nana* egg is 30 to 47 μm in diameter and has a thin shell. The oncosphere (O) is separated from the shell and contains six hooks (H). Another distinguishing feature is the presence of between four and eight filaments (F) arising from either end of the oncosphere.
The *T. solium* life cycle is similar to *T. saginata*, but the host is pork, not beef, so human infection occurs when undercooked pork is eaten. If eggs are ingested, a juvenile form called a cysticercus develops. Cysticerci may be found in any tissue, especially subcutaneous connective tissues, eyes, brain, heart, liver, lungs, and coelom. Symptoms of cysticercosis depend on the tissue infected, but mostly they are not severe. However, death of a cysticercus can produce a rapidly fatal inflammatory response. As with *T. saginata*, diagnosis to species is impossible with only the eggs; specific identification requires a scolex or gravid proglottid (Figures 11-54 and 11-55).

**Nematode Parasites Found in Clinical Specimens**

**Ascaris lumbricoides**

*Ascaris lumbricoides* (Figures 11-56 and 11-57) is a large nematode—females may reach a length of 49 cm! Human infection occurs when eggs in fecally contaminated soil or food are ingested. Juveniles emerge in the intestine, penetrate its wall, and then migrate to the lungs and other tissues. After a period of development in the lungs, the juveniles move up the respiratory tree to the esophagus and are swallowed again. Adults then reside in the small intestine and produce eggs (Figures 11-58 and 11-59). Infection may result in inflammation in organs other than the lungs where juvenile worms settled incorrectly. *Ascaris* pneumonia occurs in heavy infections due to the lung damage caused by the juveniles.
**Figure 11-56** *Ascaris lumbricoides* Anterior
*A. lumbricoides* has a cylindrical shape with three prominent mouth parts (see inset).

**Figure 11-57** *Ascaris lumbricoides* Adult Worms
*A. lumbricoides* males (bottom) are shorter than females (up to 31 cm vs. 35 cm) and have a curved posterior.

**Figure 11-58** Fertile *Ascaris lumbricoides* Fertile Egg in Feces (X1000, D’Antoni’s Iodine Stain)
Fertile *Ascaris lumbricoides* eggs are 55 to 75 μm long and 35 to 50 μm wide and are embryonated. Their surface is covered by small bumps called mammillations.

**Figure 11-59** Infertile *Ascaris lumbricoides* Egg in Feces (X1000, D’Antoni’s Iodine Stain)
Infertile eggs are longer (up to 90 μm) than fertile eggs. There is no embryo inside.

**Figure 11-60** *Enterobius vermicularis* Adult Female
Female pinworms are about 1 cm long and have a pointed tail (T) from which this group derives its common name—pinworm. Males are about half that size and have a hooked tail.

**Figure 11-61** *Enterobius vermicularis* Egg (X1000, D’Antoni’s Iodine Stain)
The eggs of *Enterobius vermicularis* are 50 to 60 μm long and 20 to 40 μm wide with one side flattened (arrow). They are usually embryonated in typical preparations.

If secondary bacterial infections occur, the pneumonia can be fatal. Blockage of the intestines and malnutrition also are possible in heavy infections. Lastly, under certain conditions, worms can wander to other body locations and cause damage or blockage. Identification of an *Ascaris* infection is made by observing the eggs in feces.

**Enterobius vermicularis**
*Enterobius vermicularis* (Figure 11-60) is the human pinworm. It is found worldwide and is especially prevalent among people in institutions (such as orphanages and mental hospitals) because conditions favor fecal-oral transmission of the parasite. Bedding, clothing, and the fingers (from scratching) become contaminated and may be involved in transmission. Poor sanitary habits of children make them especially prone to infecting others. Transmission may also involve eggs (Figure 11-61) being carried on air currents and then inhaled by a susceptible host. After ingestion, eggs hatch in the duodenum and mature in the large intestine where the adults reside. Adult females emerge from the anus at night to lay between 4,600 and 16,000 eggs in the perianal region. About one-third of pinworm infections are asymptomatic.
The other two-thirds usually do not produce serious symptoms. Diagnosis is made by identifying the eggs. Since the eggs are laid externally, they are rarely found in feces. Instead, they are collected on cellophane tape from the perianal region and examined microscopically.

**Hookworms (Ancylostoma duodenale and Necator americanus)**

The hookworm *Ancylostoma duodenale* (Figure 11-62) and *Necator americanus* (Figure 11-63) have very similar morphologies and life cycles, and the eggs are indistinguishable, so they are considered together here. Infection occurs when juveniles penetrate the skin, enter the blood and travel to the lungs. They penetrate the respiratory membrane and are carried up and out of the lungs by ciliary action to the pharynx, where they are swallowed. When they reach the small intestine, they attach and mature into adults that feed on blood and tissues of the host. Adults are rarely seen as they remain attached to the intestinal mucosa. Eggs (Figures 11-64) are passed in the feces and are diagnostic of infection. The severity of hookworm disease symptoms is related to the parasite load, and most infections are asymptomatic. As a rule, severe symptoms of bloody diarrhea and iron deficiency anemia are only seen in acute heavy or chronic infections.

**Strongyloides stercoralis**

*Strongyloides stercoralis* is the intestinal threadworm. Infection occurs by penetration of the skin by infective juveniles from fecally contaminated soil. The juveniles then migrate to the lungs and develop into parthenogenetic females that migrate to the pharynx, are swallowed, and then burrow into the intestinal mucosa. Each day they release a few dozen eggs that develop

**FIGURE 11-62** ANTERIOR OF ANCYLOSTOMA DUODENALE (X100)

*Ancylostoma duodenale* head showing the mouth and thick-walled esophagus. The bend in the head gives this group its common name—hookworm. In the inset, the chitinous teeth (arrow) are visible (compare with Figure 11-63).

**FIGURE 11-63** NECATOR AMERICANUS HEAD (X160)

This detail of *N. americanus* shows the cutting plates (CP) that help to differentiate it from *A. duodenale* (compare with Figure 11-62). Also notice the hooked head.

**FIGURE 11-64** HOOKWORM EGG IN FECES (X1000, D’ANTONI’S IODINE STAIN)

Hookworm eggs are 55 to 75 μm long and 36 to 40 μm wide. They have a thin shell and contain a developing embryo (seen here at about the 16 cell stage) that is separated from the shell when seen in fecal samples.
into juveniles (Figure 11-65) before they are passed in the feces. These juveniles may become infective or may follow a developmental path that produces free-living adults. These adults eventually produce more infective juveniles and the cycle is completed. Symptoms of infection may be itching or secondary bacterial infection at the site of entry by the infective juveniles, a cough and burning of the chest during the pulmonary phase, and abdominal pain and perhaps septicemia during the intestinal phase. Diagnosis is by finding rhabditiform larvae in fresh fecal samples.

**Wuchereria bancrofti**

*Wuchereria bancrofti* is a filarial worm that causes lymphatic filariasis. Infection occurs from the bite of a mosquito harboring infective juveniles. Upon injection into the host, the worms migrate into the large lymphatic vessels of the lower body and mature. Adults are found in coiled bunches and the females release microfilariae (Figure 11-66) by the thousands. Microfilariae enter the blood and circulate there, often with a daily periodicity—most abundant at night when the mosquito vector is active and hidden away in lung capillaries during the day when it is hot. Some infections are asymptomatic, whereas others result in acute inflammation of lymphatics associated with fever, chills, tenderness, and toxemia. In the most serious cases, obstruction of lymphatic vessels occurs and results in elephantiasis, a disease caused by accumulation of lymph fluid in the tissues, an accumulation of fibrous connective tissue, and a thickening of the skin. Diagnosis of infection is made by identifying microfilariae in blood smears.

**Materials**

- Prepared slides of:
  - *Ascaris lumbricoides* eggs in a fecal smear
  - *Clonorchis sinensis* in a fecal smear
  - *Dipylidium caninum* eggs in a fecal smear
  - *Echinococcus granulosus* hydatid cyst in section
  - *Enterobius vermicularis* eggs in a fecal smear
  - Hookworm (*Ancylostoma duodenale or Necator americanus*) eggs in a fecal smear
  - *Hymenolepis nana* eggs in a fecal smear
  - *Paragonimus westermani* eggs in a fecal smear
  - *Schistosoma mansoni* eggs in a fecal smear
  - *Strongyloides stercoralis* rhabditiform larva in a fecal smear
  - *Taenia solium* proglottid (whole mount)
  - *Taenia solium* scolex (whole mount)
  - *Taenia spp.* eggs in a fecal smear
  - *Wuchereria bancrofti* microfilariae in a blood smear

**Procedure**

Observe the prepared slides provided of the helminth specimens in fecal smears and other tissues. Scanning on low power (10x objective) is best for most preparations, then move to high-dry or oil immersion to see detail. Most egg specimens are in fecal smears, so there will be
References