proper personal hygiene, adequate sanitation practices, and avoidance of unprotected sex, particularly among homosexual men.

Quick Quiz! 7-5

All the following are highly recommended when processing samples for the identification of *Isospora belli* to ensure identification except: (Objective 7-8)

A. Iodine wet prep
B. Decreased microscope light level
C. Modified acid-fast stain
D. Saline wet prep

Quick Quiz! 7-6

Which stage of reproduction is considered capable of initiating another infection of *Isospora belli*? (Objectives 7-5)

A. Sporozoites
B. Immature oocysts
C. Merozoites
D. Mature oocysts

Quick Quiz! 7-7

Which of the following patients would be more likely to contract an infection with *Isospora belli*? (Objective 7-6)

A. HIV-positive man
B. Female leukemia patient
C. Pig farmer
D. Nursing home resident

Sarcocystis species
(sahr″ko-sis-tis)

Common associated disease and condition names: *Sarcocystis* infection.

There are a number of species of parasites that fall within the group known as *Sarcocystis*. Cattle may harbor *Sarcocystis hovihominis*, also known as *Sarcocystis hominis*. Similarly, *Sarcocystis suihominis* may be found in pigs. In addition to these typical farm animals, a variety of wild animals may also harbor members of the *Sarcocystis* group. *Sarcocystis lindemanni* has been designated as the umbrella term for those organisms that may potentially parasitize humans.

Morphology

Mature Oocysts. Members of the genus *Sarcocystis* were originally classified and considered as members of the genus *Isospora*, in part because of the striking morphologic similarities of these parasites (Fig. 7-8; Table 7-4). The oval transparent organism consists of two mature sporocysts that each average from 10 to 18 μm in length. Each sporocyst is equipped with four sausage-shaped sporozoites. A double-layered clear and colorless cell wall surrounds the sporocysts.

Laboratory Diagnosis

Stool is the specimen of choice for the recovery of *Sarcocystis* organisms. The oocysts are usually passed into the feces fully developed. When present, these mature oocysts are typically seen

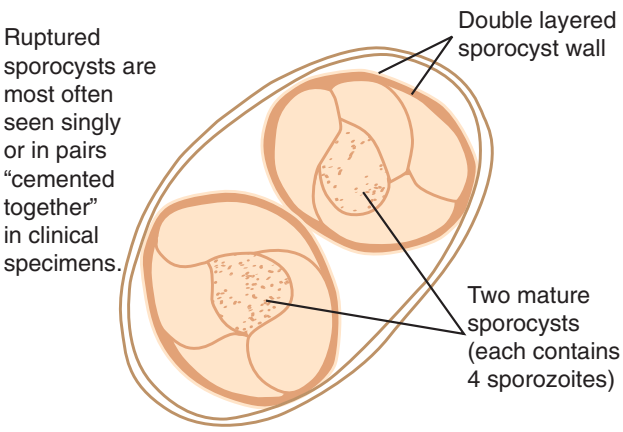

FIGURE 7-8 *Sarcocystis species* oocyst.

TABLE 7-4 *Sarcocystis* spp. Mature Oocyst: Typical Characteristics at a Glance*

Parameter	Description
Shape	Oval
Appearance	Transparent
Number of sporocysts	Two
Size of each sporocyst	10-18 µm long
Contents of each sporocyst	Four sausage-shaped sporozoites
Oocyst cell wall appearance	Clear, colorless, double layered

*In many cases, only single or double sporocysts cemented together may be visible in stool samples.

in wet preparations. However, in many cases, the oocysts have already ruptured and only the sporocysts are visible on examination of the stool specimen. The sporocysts may be seen singly or in pairs that appear to be cemented together. Routine histologic methods may be used to identify the *Sarcocystis* cyst stage, known as the sarcocyst, from human muscle samples. An in-depth discussion of these histologic methods is beyond the scope of this text.

Life Cycle Notes

Although the morphology of the oocysts of *Sarcocystis* resembles that of *Isospora*, the life cycles of these two genera are different—hence, the current organism classification. Asexual reproduction of *Sarcocystis* occurs in the intermediate host. Human infection of *Sarcocystis* species may be initiated in one of two ways. The first transmission route occurs when uncooked pig or cattle meat infected with *Sarcocystis* sarcocysts is ingested. Humans are the definitive host. Gametogony usually occurs in the human intestinal cells. The development of oocysts and subsequent release of sporocysts thus follow. This sets the stage for continuation of the life cycle in a new intermediate host. The second transmission route occurs when humans accidentally swallow oocysts from stool sources of animals other than cattle or pigs. In this case, the ingested sarcocysts take up residence in human striated muscle. Under these circumstances, the human serves as the intermediate host. It is interesting to note that *Sarcocystis* oocysts do not infect the host of their origin.

Epidemiology

The frequency of *Sarcocystis* infections is relatively low, even though its distribution is worldwide. In addition to its presence in cattle and pigs, *Sarcocystis* spp. may also be found in a variety of wild animals.

Clinical Symptoms

***Sarcocystis* Infection.** There have only been a few documented symptomatic cases of *Sarcocystis* infections in compromised patients. These persons experienced fever, severe diarrhea, weight loss, and abdominal pain. It is presumed that patients suffering from muscle tenderness and other local symptoms are exhibiting symptoms caused by *Sarcocystis* invasion of the striated muscle.

Treatment

The treatment protocol for infections with *Sarcocystis* spp. when humans are the definitive host is similar to that for *Isospora belli*. The combined medications of trimethoprim plus sulfamethoxazole or pyrimethamine plus sulfadiazine are typically given to treat these infections. There is no known specific chemotherapy to treat *Sarcocystis* infections of the striated muscle when humans are the intermediate host.

Prevention and Control

The primary prevention and control measures of *Sarcocystis* infections in which humans are the definitive host consist of adequate cooking of beef and pork. Prevention of those infections in which humans are the intermediate host includes the proper care and disposal of animal stool that may be potentially infected with *Sarcocystis*.

Quick Quiz! 7-8

Which genus of parasite is most similar to *Sarcocystis* based on morphologic similarities? (Objective 7-11)

A. *Isospora*
B. *Blastocystis*
C. *Entamoeba*
D. *Toxoplasma*

Quick Quiz! 7-9

How do humans become infected with *Sarcocystis*? (Objectives 7-5)

A. Ingestion of uncooked or undercooked beef or pork
B. Inhalation of oocysts
C. Ingestion of animal fecal contaminated food
D. More than one of the above: _____ (specify)

Quick Quiz! 7-10

In addition to oocysts, these *Sarcocystis* morphologic forms may be seen in human samples: (Objective 7-5)

A. Packets of eggs
B. Single or double sporocysts
C. Clusters of cysts
D. Groups of sporoblasts

Cryptosporidium parvum
(krip"toe-spor-i'dee-um/par-voom)

Common associated disease and condition names: Cryptosporidosis.

Morphology

Oocysts. Measuring only 4 to 6 µm, the roundish *Cryptosporidium* oocysts are often confused with yeast (Figs. 7-9 and 7-10; Table 7-5). Although not always visible, the mature oocyst consists of four small sporozoites surrounded by a thick cell wall. Contrary to other members of the sporozoa, such as *Isospora*, *Cryptosporidium* oocysts do not contain sporocysts. One to six dark granules may also be seen.

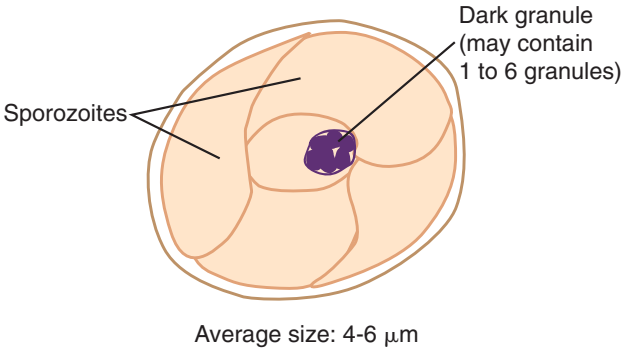

FIGURE 7-9 *Cryptosporidium parvum* oocyst.

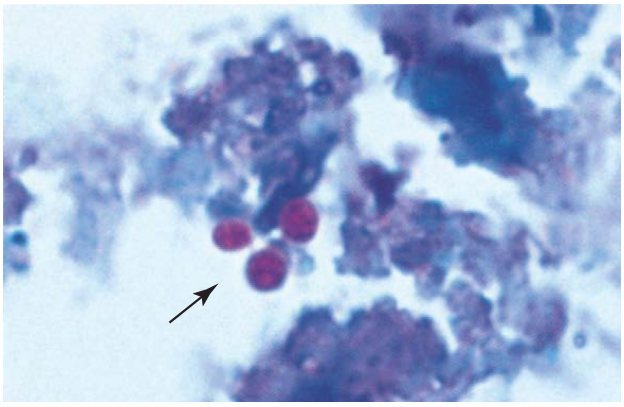

FIGURE 7-10 Modified acid-fast stain, ×1000). Arrows indicate *Cryptosporidium oocysts*, each containing four undefined sporozoites. Note dark-staining granules.

TABLE 7-5 *Cryptosporidium parvum* Oocyst: Typical Characteristics at a Glance

Parameter	Description
Size	4-6 µm
Shape	Roundish
Number of sporocysts	None
Number of sporozoites	Four (small)
Other features	Thick cell wall One to six dark granules may be visible

Schizonts and Gametocytes. The other morphologic forms required to complete the life cycle of *Cryptosporidium* include schizonts containing four to eight merozoites, microgametocytes, and macrogametocytes. The average size of these forms is a mere 2 to 4 μm. It is important to note that these morphologic forms are not routinely seen in patient samples.

Laboratory Diagnosis

The specimen of choice for the recovery of *Cryptosporidium* oocysts is stool. Several methods have been found to identify these organisms successfully. The oocysts may be seen using iodine or modified acid-fast stain. In addition, formalin-fixed smears stained with Giemsa may also yield the desired oocysts. As noted, it is important to distinguish yeast (Chapter 12) from true oocysts. Oocysts have also been detected using the following methods: the Enterotest, enzyme-linked immunosorbent assay (ELISA), and indirect immunofluorescence. Concentration via modified zinc sulfate flotation or by Sheather's sugar flotation have also proven successful, especially when the treated sample is examined under phase contrast microscopy. It is important to note that merozoites and gametocytes are usually only recovered in intestinal biopsy material.

Life Cycle Notes

Cryptosporidium infection typically occurs following ingestion of the mature oocyst. Sporozoites emerge after excystation in the upper gastrointestinal tract, where they take up residence in the cell membrane of epithelial cells. Asexual and sexual multiplication may then occur. Sporozoites rupture from the resulting oocysts and are capable of initiating an autoinfection by invading new epithelial cells. A number of the resulting oocysts remain intact, pass through the feces, and serve as the infective stage for a new host.

It is interesting to note that two forms of oocysts are believed to be involved in the *Cryptosporidium* life cycle. The thin-shelled version is most likely responsible for autoinfections because it always seems to rupture while still inside the host. The thick-shelled oocyst usually remains intact and is passed out of the body. This form is believed to initiate autoinfections only occasionally.

Epidemiology

Cryptosporidium has worldwide distribution. Of the 20 species known to exist, only *C. parvum* is known to infect humans. Infection appears to primarily occur by water or food contaminated with infected feces, as well as by person-to-person transmission. Immunocompromised persons, such as those infected with the AIDS virus, are at risk of contracting this parasite. Other populations potentially at risk include immunocompetent children in tropical areas, children in day care centers, animal handlers, and those who travel abroad.

Clinical Symptoms

Cryptosporidiosis. Otherwise healthy persons infected with *Cryptosporidium* typically complain of diarrhea, which is self-limiting and lasts approximately 2 weeks. Episodes of diarrhea lasting 1 to 4 weeks have been reported in some day care centers. Fever, nausea, vomiting, weight loss, and abdominal pain may also be present. When fluid loss is great because of the diarrhea and/or severe vomiting, this condition may be fatal, particularly in young children.

Infected immunocompromised individuals, particularly AIDS patients, usually suffer from severe diarrhea and one or more of the symptoms described earlier. Malabsorption may also accompany infection in these patients. In addition, infection may migrate to other body areas, such as the stomach and respiratory tract. A debilitating condition that leads to death may result in these patients. Estimated infection rates in AIDS patients range from 3 to 20% in the United States and 50 to 60% in Africa and Haiti. *Cryptosporidium* infection is considered to be a cause of morbidity and mortality.

Treatment

Numerous experiments to treat *Cryptosporidium* using a wide variety of medications have been conducted. Unfortunately, most of these potential treatments have proven ineffective. However, the use of spiramycin, even though still in the experimental stage, has preliminarily proven helpful in ridding the host of *Cryptosporidium*. More research on this treatment and on the newer antiparasitic medications are necessary to develop effective medications.

Prevention and Control

Proper treatment of water supplies, handling known infected material by using gloves and wearing a gown (when appropriate), proper hand washing, and properly disinfecting potentially infected equipment with full-strength commercial bleach or 5% to 10% household ammonia are crucial to the prevention and control of *Cryptosporidium*. In addition, enteric precautions should be observed when working with known infected persons.

Notes of Interest and New Trends

Cryptosporidium spp. were first associated with poultry and cattle. *C. parvum* is now recognized as the agent responsible for neonatal diarrhea in calves and lambs, a life-threatening condition.

Human *Cryptosporidium* infection was first reported in 1976. The first cases were isolated from persons with compromised immune systems and were considered infrequent in occurrence.

Several outbreaks in the public water supply were attributed to contamination of *Cryptosporidium* oocysts. This occurred in Carroll County, Georgia in 1987. More recently in June 2011, and Indiana fire station reported gastrointestinal illness in a substantial percentage of their workers who had extinguished a barn fire on a nearby Michigan farm. An investigation by the Michigan Department of Community Health revealed that the firefighters used local hydrant water and on site swimming pond water to extinguish the fire. The pond water was discovered to be contaminated with **Cryptosporidium** from calf feces.

A modification of the standard stool processing technique (see Chapter 2), which includes layering and flotation of the sample over a hypertonic sodium chloride solution, has successfully separated *Cryptosporidium* oocysts from fecal debris.

Quick Quiz! 7-11

Which stage of reproduction is considered capable of autoinfection of *Cryptosporidium*? (Objectives 7-5)

A. Intact oocysts
B. Merozoites
C. Gametocytes
D. Sporozoites

Quick Quiz! 7-12

The permanent stain of choice for the recovery of *Cryptosporidium parvum* is: (Objective 7-8)

A. Iron hematoxylin
B. Modifed acid-fast
C. Gram
D. Trichrome

Quick Quiz! 7-13

All the following are recommended to prevent and control an outbreak of *Cryptosporidium* except: (Objective 7-7C)

A. Proper treatment of water supplies
B. Sterlize equipment using high heat.
C. Sterilize equipment using full-strength bleach.
D. Sterilize equipment using 5% to 10% household ammonia.

Blastocystis hominis

(blas'toe-sis-tis/hom'i-nis)

Common associated disease and condition names: *Blastocystis hominis* infection.

Morphology

Although a number of different morphologic forms of *B. hominis* are known to exist, the most

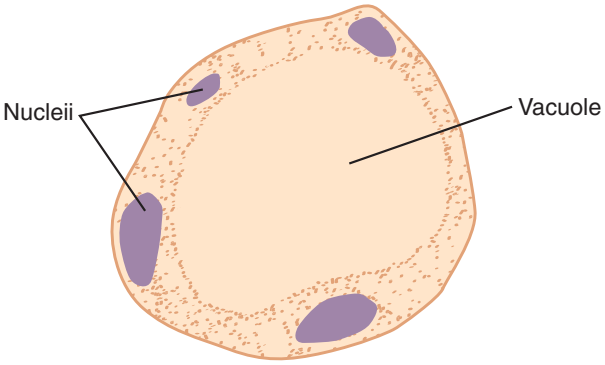

FIGURE 7-11 *Blastocystis hominis* vacuolated form.

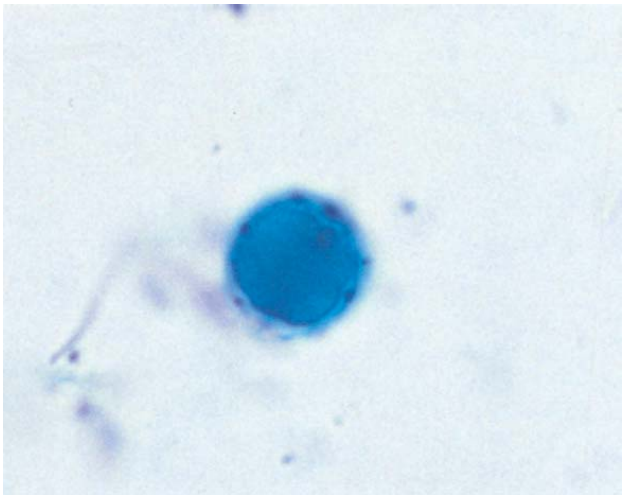

FIGURE 7-12 Trichrome stain, 1000×. Typical *Blastocystis hominis* vacuolated form.

common form seen and the easiest to recognize is the vacuolated form. Therefore, only this form will be described here.

Vacuolated Forms. Although the vacuolated form of *B. hominis* may range in size from 5 to 32 μm, the average form measures only 7 to 10 μm (Figs. 7-11 and 7-12; Table 7-6). This morphologic form is characterized by a large, central, fluid-filled vacuole that consumes almost 90% of the cell. The remaining 10% assumes the periphery of the organism; it consists of a ring of cytoplasm in which two to four nuclei are typically present.

TABLE 7-6 ***Blastocystis hominis* Vacuolated Form: Typical Characteristics at a Glance**

Parameter	Description
Size	5-32 μm
Vacuole	Centrally located Fluid-filled structure Consumes almost 90% of organism
Cytoplasm	Appears as ring around periphery of organism
Nuclei	Two to four located in cytoplasm

Laboratory Diagnosis

Stool is the specimen of choice for the recovery of *Blastocystis*. In iodine wet preparations, the peripheral cytoplasm containing one or more nuclei appears a light yellow in color, whereas the central vacuole does not stain and appears clear and transparent. In permanent stain preparations, the central vacuole may vary in its ability to stain from not at all to very apparent. The nuclei located in the peripheral cytoplasm in these preparations typically stain dark. It is important to note here that saline, like water, usually lyses this organism and may lead to a false-negative result. Therefore, it is important to screen suspicious samples with an iodine wet preparation and to use a permanent stain to confirm the presence of the parasite.

Life Cycle Notes

B. hominis reproduces by sporulation or binary fission. The organism passes through a number of morphologic forms during these processes. *B. hominis* participates in sexual and asexual reproduction, and exhibits pseudopod extension and retraction. A detailed discussion of the *B. hominis* life cycle has not been widely presented.

Epidemiology

Early nonscientific documentation of *B. hominis* infections indicated that they occurred as epidemics in subtropical countries. Select articles on *B. hominis* over the past 10 to 25 years or so suggest that this organism may be found in a

number of climates worldwide, ranging from Saudi Arabia to British Columbia. The results of one study conducted in Saudi Arabia were inconclusive regarding whether travel is a risk factor in contracting this parasite. Infection of *B. hominis* is initiated by ingestion of fecally contaminated food or water.

Clinical Symptoms

***Blastocystis hominis* Infection.** The pathogenicity of *B. hominis* is not totally clear, although the symptoms have been defined. Patients who suffer from infection with *B. hominis* in the absence of other intestinal pathogens (including parasites, bacteria, and viruses) may experience diarrhea, vomiting, nausea, and fever, as well as abdominal pain and cramping. Thus, *B. hominis* might be considered a pathogen. However, it has also been suggested that these patients may have an additional undetected pathogen that is ultimately responsible for the discomfort.

In persons infected with *B. hominis* in addition to another pathogenic organism (e.g., *E. histolytica, Giardia intestinalis*), it is this underlying agent that is thought to be the pathogen. These patients usually experience severe symptoms, as described earlier.

Treatment

Iodoquinol or metronidazole is recommended for the treatment of *B. hominis*. This has been suggested for patients infected with *Blastocystis* who have no other obvious reason for their diarrhea.

Prevention and Control

Proper treatment of fecal material, thorough hand washing, and subsequent proper handling of food and water are critical to halt the spread of *Blastocystis*.

Notes of Interest and New Trends

Blastocystis hominis was given its current name in 1912 by Emile Brumpt.

Since its discovery, *B. hominis* has been the subject of controversy. Initially, the organism was considered as an algae, then as a harmless intestinal yeast, and as a protozoan parasite since the 1970s. Genetic analyses in 1996 showed that Blastocystis is not fungal or protozoan. Since then, its classification has undergone major reviews which definitely place it into Stramenopiles, a major line of eukaryotes.

Quick Quiz! 7-14

Which is the best screening method for the identification of *Blastocystis hominis*? (Objective 7-8)

A. Saline wet prep
B. Modified acid-fast stain
C. Iodine wet prep
D. Iron hematoxylin stain

Quick Quiz! 7-15

Blastocystis hominis is always considered as being responsible for clinical symptoms when present in human samples. (Objective 7-6)

A. True
B. False

Quick Quiz! 7-16

Which of the following measures that when taken can prevent the spread of *Blastocystis hominis*? (Objective 7-7C)

A. Avoid swimming in potentially contaminated water.
B. Proper sewage treatment
C. Use insect repellent.
D. Avoid unprotected sex.

Cyclospora cayetanensis

(si'klō-spor-uh)

Common associated disease and condition names: *Cyclospora cayetanensis* infection.

Morphology

Oocysts. *Cyclospora cayetanensis* infection is similar to cryptosporidiosis (Table 7-7). It is an

TABLE 7-7 *Cyclospora cayetanensis* Mature Oocyst: Typical Characteristics at a Glance

Parameter	Description
Size	7-10 µm in diameter
Number of sporocysts	Two
Contents of sporocysts	Each sporocyst contains two sporozoites

intestinal coccidial organism. Infected patients shed oocysts that measure 7 to 10 µm in diameter and, on maturation, form two sporocysts, each containing only two sporozoites.

Laboratory Diagnosis

Diagnosis of *C. cayetanensis* may be accomplished when stool samples are concentrated nontraditionally without the use of formalin fixative. *C. cayetanensis* oocysts sporulate best at room temperature. The addition of 5% potassium dichromate allows the sporocysts to become visible. Flotation methods followed by examination using the preferred phase contrast or bright field microscopy have also proven successful in isolating *C. cayetanensis*. Modified acid-fast stain may also be used to detect the oocysts. Oocysts autofluoresce under ultraviolet light microscopy.

Life Cycle Notes

The life-cycle of *C. cayetanensis*, like that of *Isospora,* begins with ingestion of an oocyst. The oocyst contains two sporocysts, each enclosing two sporozoites. Once inside a human host, cells in the small intestine provide a suitable environment for the emergence of sporozoites. The sporozoites undergo asexual reproduction, producing numerous merozoites, as well as sexual development, resulting in macrogametocyte and microgametocyte production. Male and female gametocytes unite and form oocysts. Infected humans pass immature oocysts in the stool. Under optimal conditions, these oocyts continue to develop and mature outside the human body, a process that may take 1 or more weeks to complete. Once the maturation process is complete, the resultant oocysts are capable of initiating a new cycle. No animal reservoir exists.

Epidemiology

C. cayetanensis infections are known to occur in many countries, including the United States and Canada. Furthermore, cases of infection caused by *C. cayetanensisa* have been reported in children living in unsanitary conditions in Lima, Peru, as well as in travelers and foreigners residing in Nepal and parts of Asia. Contaminated water in Chicago presumably was the source of a minioutbreak in 1990 that occurred in a physician's dormitory. Contaminated lettuce and fresh fruit (raspberries have been known to be a source of infection), often imported, have also been associated with *C. cayetanensis* infections.

Clinical Symptoms

***Cyclospora cayetanensis* Infection.** The clinical symptoms associated with *C. cayetanensis* infections in children are similar to those seen in cases of cryptosporidiosis. The notable difference among infections caused by these two organisms in adults is that *C. cayetanensis* produces a longer duration of diarrhea. There is no known connection between *C. cayetanensis* infection and immunocompromised patients.

Prevention and Control

Prevention and control measures associated with *C. cayetanensis* consist of properly treating water prior to use and only using treated water when handling and processing food.

Notes of Interest and New Trends

It appears that this parasite may not be recovered using standard or traditional specimen processing techniques. The alternative techniques discussed in the laboratory diagnosis section may

be necessary for samples suspected of containing *C. cayetanensis* in the future.

Quick Quiz! 7-17

Diagnosis of *Cyclospora* can be accomplished by all the following except: (Objective 7-8)
A. Concentration with formalin fixative
B. Flotation methods
C. Modified acid-fast stain
D. Addition of 5% potassium dichromate

Quick Quiz! 7-18

The clinical symptoms associated with *Cyclospora* infections in children are similar to those seen in cases of infection by which of the following? (Objectives 7-11)
A. Naegleria
B. Cryptosporidium
C. Leishmania
D. Balantidium

Quick Quiz! 7-19

The most important *Cyclospora* prevention step that can be taken is: (Objective 7-7C)
A. Proper water treatment
B. Wearing shoes when walking in sandy soil
C. Insecticide treatment of mosquito breeding areas
D. Thoroughly cooking beef and pork.

Microsporidia
(mi'kro-spor-i'dee-uh)

Common associated disease and condition names: Microsporidia infection, microsporidial infection.

Although it is classified as a protozoal disease by the World Health Organization, Microsporidia's phylogenetic placement has been resolved within the Fungi as a result of DNA testing. There are a number of genera and species of parasites that are members of the phylum Microsporidia. Three of the five genera known to cause human disease have been reported in patients suffering from AIDS. The most well-known member is *Enterocytozoon bieneusi,* which causes enteritis in these patients. Species of *Encephulitozoon* and *Pleistophora* have also been described as infecting AIDS patients and causing severe tissue infections. Of the remaining two genera, *Microsporidium* is noted for corneal infections, as well as *Nosema*. In addition, *Nosema* produced a fatal infection in a severely immunocompromised infant.

Morphology

Spores. Although it has been documented that there are a number of different morphologic forms, spores are the only ones that have been well described (Table 7-8). These spores are very small, ranging in size from 1 to 5 µm. Unlike the other protozoa, Microsporidia spores are characteristically equipped with extruding polar filaments (tubules), which initiate infection by injecting sporoplasm (infectious material) into a host cell.

Laboratory Diagnosis

Diagnosis of the different species of Microsporidia varies. Serologic tests are available for the detection of some species. In addition, some species will grow in cell culture. A number of stains may be used to detect all or part of the spore microscopically. Thin smears stained with trichrome or acid-fast stain may show the desired spores. Microsporidia stain gram-positive and show partial positive staining when treated with acid-fast stain or the histologic

TABLE 7-8 *Microsporidia* Spore: Typical Characteristics at a Glance

Parameter	Description
Size	1-5 µm
Other features	Equipped with extruding polar filaments (or tubules) that initiate infection by injecting sporoplasm (infectious material) into host cell

stain periodic acid-Schiff (PAS). Giemsa-stained biopsy material and fecal concentrate specimens readily show the spores. It is important to note that speciation of the Microsporidia requires the use of transmission electron microscopy. Molecular diagnostic methods are being developed.

Life Cycle Notes

Transmission of Microsporidia may be direct or may involve an intermediate host. On entering the host, human infection is initiated when the infective spores inject sporoplasm into a host cell. A complex reproductive process occurs, new spores emerge, and additional cells typically become infected. Spores are dispersed into the outside environment in the direct transmission cycle in the feces or urine, or by the death of the host. In addition, the spores may be ingested by a carnivorous animal.

Epidemiology

Cases of *E. bieneusi* infection have been reported in AIDS patients from Haiti, Zambia, Uganda, the United Kingdom, the United States, and the Netherlands. Although most documented infections of Microsporidia parasites have occurred in AIDS patients, cases in persons with normal immune systems have also been described.

Clinical Symptoms

Microsporidial Infection. Patients suffering from infections with Microsporidia have been known to develop enteritis, keratoconjunctivitis, and myositis. Infections involving peritonitis and hepatitis have rarely occurred.

Treatment

Albendazole is recommended for the treatment of *E. bieneusi;* oral fumagillin is recommended as an alternative treatment. Albendazole plus fumagillin eye drops are recommended for the treatment of *Nosema* infection.

Notes of Interest and New Trends

Persons infected with *C. cayetanensis* in addition to Microsporidia have been reported and are considered somewhat common.

In recent years, the United States Environmental Protection Agency (EPA) has listed Microsporidia in the EPA Candidate Contaminate List, deeming it an emerging water-borne pathogen needing monitorial attention.

Although Microsporidia infection in humans mostly occurs in immunocompromised patients, the further spread of AIDS worldwide increases our need to understand and manage Microsporidia for the near future.

Quick Quiz! 7-20

How do *Microsporidia* spores differ from other protozoan spores? (Objective 7-11)

A. Double outer wall
B. Extruding polar filaments
C. Cilia
D. Pseudopods

Quick Quiz! 7-21

Of the following, which laboratory technique is required for species identification of Microsporidia? (Objective 7-8)

A. Giemsa-stained biopsy material
B. Electron microscopy
C. Fecal concentration
D. PAS stain

Quick Quiz! 7-22

The life cycle of Microsporidia is a complex process in which both the infective and diagnostic stages are spores. (Objective 7-5)

A. True
B. False

Toxoplasma gondii

(tock″so-plaz′muh/gon′dee-eye)

Common associated disease and condition names: Toxoplasmosis, congenital toxoplasmosis, cerebral toxoplasmosis.

Morphology

There are only two morphologic forms of trophozoites seen in humans, tachyzoites and bradyzoites. The infective form for humans is the oocyst. This form may be encountered on occasion, especially where veterinary parasitologic techniques are performed. Thus, all three of these morphologic forms are discussed in this section.

Oocyst. The typical infective form of *Toxoplasma gondii*, the oocyst, is similar in appearance to that of *Isospora belli*. The most notable difference between the two organisms is that *T. gondii* is smaller. The round to slightly oval form measures 10 to 15 µm long by 8 to 12 µm wide. The transparent oocyst contains two sporocysts, each with four sporozoites. The organism is bordered by a clear, colorless, two-layered cell wall.

Tachyzoites. The actively multiplying, crescent-shaped tachyzoites range in size from 3 to 7 µm by 2 to 4 µm (Fig. 7-13; Table 7-9). One end of the organism often appears more rounded than the other end. Each tachyzoite is equipped with a single centrally located nucleus, surrounded by a cell membrane. A variety of other organelles are present, including a mitochondrion and Golgi apparatus; however, these structures are not readily visible.

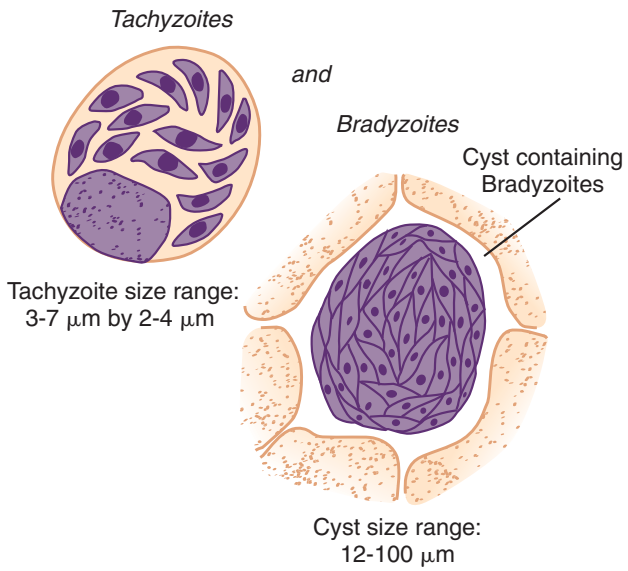

FIGURE 7-13 *Toxoplasma gondii* tachyzoites and bradyzoites.

Bradyzoites. Although there is evidence to support an antigenic difference, the typical bradyzoite basically has the same physical appearance as the tachyzoite, only smaller (see Fig. 7-13; Table 7-10). These slow-growing viable forms gather in clusters inside a host cell, develop a surrounding membrane, and form a cyst in a variety of host tissues and muscles outside the intestinal tract. Such cysts may contain as few as 50 and up to as many as several thousand bradyzoites. A typical cyst measures from 12 to 100 µm in diameter.

Laboratory Diagnosis

The primary means of diagnosing *T. gondii* infections is analyzing blood samples using serologic

TABLE 7-9 ***Toxoplasma gondii* Tachyzoites: Typical Characteristics at a Glance**

Parameter	Description
General comment	Actively multiplying morphologic form
Size	3-7 × 2-4 µm
Shape	Crescent-shaped, often more rounded on one end
Number of nuclei	One
Other features	Contains a variety of organelles that are not readily visible

TABLE 7-10 ***Toxoplasma gondii* Bradyzoites: Typical Characteristies at a Glance**

Parameter	Description
General comment	Slow-growing morphologic form
Size	Smaller than tachyzoites
Physical appearance	Similar to that of the tachyzoites
Other features	Hundreds to thousands of bradyzoites enclose themselves to form a cyst that may measure 12-100 µm in diameter

test methods. The recommended test for the determination of immunoglobulin M (IgM) antibodies present in congenital infections is the double-sandwich ELISA method. Both IgM and IgG levels may be determined using the indirect fluorescent antibody (IFA) test. Additional serologic tests for the IgG antibody include the indirect hemagglutination (IHA) test and ELISA. The actual demonstration of *T. gondii* trophozoites (tachyzoites) and cysts (filled with bradyzoites) involves tedious microscopic examination of infected human tissue samples or the inoculation of laboratory animals. The time and effort to perform such testing is, in most cases, not practical.

Life Cycle Notes

Although the natural life cycle of *T. gondii* is relatively simple, the accidental cycle may involve a number of animals and humans. The definitive host in the *T. gondii* life cycle is the cat (or other felines). On ingestion of *T. gondii* cysts present in the brain or muscle tissue of contaminated mice or rats, the enclosed bradyzoites are released in the cat and quickly transform into tachyzoites. Both sexual and asexual reproduction occur in the gut of the cat. The sexual cycle results in the production of immature oocysts, which are ultimately shed in the stool. The oocysts complete their maturation in the outside environment, a process that typically takes from 1 to 5 days. Rodents, particularly mice and rats, serve as the intermediate hosts, ingesting the infected mature *T. gondii* oocysts while foraging for food. The sporozoites emerge from the mature oocyst and rapidly convert into actively growing tachyzoites in the intestinal epithelium of the rodent. These tachyzoites migrate into the brain or muscle of the intermediate host, where they form cysts filled with bradyzoites. The cat becomes infected on ingestion of a contaminated rodent and the cycle repeats itself.

Human infection of *T. gondii* is accidental and may be initiated in four ways. One route occurs when humans are in contact with infected cat feces and subsequently ingest the mature oocysts present via hand-to-mouth transmission. Cat litter boxes, as well as children's sandboxes, are the primary sources of such infected fecal matter.

The second route involves human ingestion of contaminated undercooked meat from cattle, pigs, or sheep. These animals, as well as a wide variety of other animals, may contract *T. gondii* during feeding by ingesting infective oocysts present in cat feces. The infective sporozoites are released following ingestion and follow the same cycle in these animals as they do in the natural intermediate hosts. The resulting cysts form in the animal muscle and the parasites within them may remain viable for years.

The third means of human *T. gondii* transfer is transplacental infection. This occurs when an asymptomatic infection in a mother is unknowingly transmitted to her unborn fetus. In response to the parasite, the mother produces IgG, which also crosses the placenta and may appear for several months in the circulation of the fetus/newborn. In addition, the mother produces IgM, which does not cross the placenta. However, the infant may demonstrate anti–*T. gondii* IgM from birth to several months old.

Although extremely rare, the fourth route of human infection occurs when contaminated blood is transfused into an uninfected person.

Once inside the human, *T. gondii* tachyzoites emerge from the ingested cyst and begin to grow and divide rapidly. The tachyzoite form is responsible for the tissue damage and initial infection. The tachyzoites migrate to a number of tissues and organs, including the brain, where cysts filled with bradyzoites then form.

Epidemiology

T. gondii is found worldwide, primarily because such a large variety of animals may harbor the organism. It appears from information collected to date that no population is exempt from the possibility of contracting *T. gondii*. One of the most important populations at risk for contracting this parasite is individuals suffering from AIDS.

There are several epidemiologic considerations worth noting. First, it has been documented that *T. gondii* infections occur in 15% to 20% of the population in the United States. Second, infection caused by the consumption of undercooked meat and its juices by women and their children in Paris was reported in 93% (the highest recorded rate) and 50%, respectively, of the local population. Third, there have been an estimated 4000 infants born with transplacentally acquired *T. gondii* infections in the United States each year. Fourth, the *T. gondii* mature oocysts are incredibly hardy and can survive for long periods under less than optimal conditions. In the state of Kansas, it was documented that these oocysts survived up to 18 months in the outside environment, withstanding two winter seasons. Finally, human infections in the United States are usually acquired by hand-to-mouth contamination of infected oocysts in cat feces, ingesting contaminated meat, or transplacentally during pregnancy. As noted, transfusion-acquired *T. gondii* may also occur; however, it is extremely rare.

There are numerous other reports of *T. gondii* infections that have occurred worldwide. However, an in-depth discussion of these epidemiologic findings is beyond the scope of this chapter.

Clinical Symptoms

Asymptomatic. Many patients infected with *T. gondii* remain asymptomatic, especially children who have passed the neonatal stage of their lives. Although well adapted to its surroundings, *T. gondii* appears to only cause disease in humans when one or more of the following conditions have been met: (1) a virulent strain of the organism has entered the body; (2) the host is in a particularly susceptible state (e.g., those suffering from AIDS); and (3) the specific site of the parasite in the human body is such that tissue destruction is likely to occur.

Toxoplasmosis: General Symptoms. Although severe symptoms may be noted, the typical symptoms experienced by individuals infected with *T. gondii* are mild and mimic those seen in cases of infectious mononucleosis. This acute form of the disease is characterized by fatigue, lymphadenitis, chills, fever, headache, and myalgia. In addition to the symptoms mentioned, chronic disease sufferers may develop a maculopapular rash as well as show evidence of encephalomyelitis, myocarditis, and/or hepatitis. Retinochoroiditis with subsequent blindness has been known to occur on rare occasions.

Congenital Toxoplasmosis. This severe and often fatal condition occurs in approximately one to five of every 1000 pregnancies. Transmission of the disease occurs when the fetus is infected (via transplacental means) unknowingly by its asymptomatic infected mother. The degree of severity of the resulting disease varies and is dependent on two factors: (1) antibody protection from the mother; and (2) the age of the fetus at the time of infection. Mild infections occur occasionally and result in what appears to be a complete recovery. Unfortunately, these patients may develop a subsequent retinochoroiditis years after the initial infection. Typical symptoms in an infected child include hydrocephaly, microcephaly, intracerebral calcification, chorioretinitis, convulsions, and psychomotor disturbances. Most of these infections ultimately result in mental retardation, severe visual impairment, or blindness.

There are a number of important documented statistics regarding the symptoms that infants born with *T. gondii* infection are likely to experience.

It is estimated that 5% to 15% of infected infants will die as a result of toxoplasmosis infection.

Another 10% to 13% of infected infants will most likely develop moderate to severe handicaps.

Severe eye and brain damage will occur in approximately 8% to 10% of infected infants.

The remaining 58% to 72% of infected infants will most likely be asymptomatic at birth.

Although the mechanism of this infection reactivation is unknown, a small percentage of these infants will develop mental retardation or

retinochoroiditis later in life, usually as children or young adults.

Toxoplasmosis in Immunocompromised Patients. Patients immunosuppressed because of organ transplantation or the presence of neoplastic disease, such as Hodgkin's lymphoma, have long been known to contract toxoplasmosis as an opportunistic infection. It is important to note, particularly in patients needing blood transfusions, the importance of screening potential donor units for toxoplasmosis prior to transfusion.

Cerebral Toxoplasmosis in AIDS Patients. A focus of attention has been the association of *T. gondii* and AIDS patients. Since the 1980s, toxoplasmic encephalitis has been considered a significant complication in these individuals. In fact, one of the first apparent clinical symptoms of patients with AIDS may be that of central nervous system (CNS) involvement by *T. gondii*. AIDS patients suffering from infection with *T. gondii* may experience early symptoms of headache, fever, altered mental status (including confusion), and lethargy. Subsequent focal neurologic deficits, brain lesions, and convulsions usually develop.

The *T. gondii* organisms do not spread into other organs of the body but rather stay confined within the CNS. A rise in spinal fluid IgG antibody levels is diagnostic, as is the demonstration of tachyzoites in the cerebrospinal fluid (CSF) on microscopic examination. The serum IgG level in these patients does not respond, nor does that of the CSF. Most infected patients do not have serum levels of IgM antibodies. The lack of serum IgM coupled with the lack of change in serum IgG levels in these patients suggests that their infections occurred because of a reactivation of a chronic latent infection and not because of an acquired primary infection.

Treatment

The treatment of choice for symptomatic cases of *T. gondii* infection consists of a combination of trisulfapyrimidines and pyrimethamine (Daraprim). It is important to note that infected pregnant women should not be given pyrimethamine. An acceptable alternative drug is spiramycin. Spiramycin is used in Europe, Canada, and Mexico but is still considered an experimental drug in the United States. However, it can be obtained by special permission from the FDA for toxoplasmosis in the first trimester of pregnancy. Corticosteroids used as an anti-inflammatory agent may also be of value. Folinic acid (leucovorin) may be administered to infected AIDS patients to counteract the bone marrow suppression caused by pyrimethamine. An effective drug, particularly for the treatment of toxoplasmic encephalitis in patients with AIDS, is atovaquone.

Prevention and Control

There are a number of measures that must be implemented and enforced to prevent the spread of *T. gondii* infections. One is the avoidance of contact with cat feces. This may be accomplished by wearing protective gloves when cleaning out a cat litter box, disinfecting the litter box with boiling water, and thorough hand washing afterward. In addition, placing a protective cover over children's sandboxes when not in use will keep cats from using them as litter boxes.

T. gondii infections may also be prevented by the avoidance of ingesting contaminated meat. This may be accomplished by thorough hand washing after handling contaminated meat, as well as the avoidance of tasting raw meat. In addition, all meat should be thoroughly cooked prior to human consumption. Additional *T. gondii* prevention and control measures include keeping cats away from potentially infective rodents, feeding cats only dry or cooked canned cat food, and/or not having cats at all.

All humans should practice these preventive measures. However, pregnant women should be especially cautious around cat feces and contaminated meat because of the possibility of contracting toxoplasmosis and transferring the disease to their unborn children.

Notes of Interest and New Trends

In 1908, the African rodent *Ctenodactylus gondii* was the first animal discovered with *T.*

gondii—hence, the name. It was not until 1939 that *T. gondii* was recognized as a cause of transplacental infections.

Techniques using the polymerase chain reaction (PCR) assay have been developed. Successful results were achieved when analyzing samples of venous blood from AIDS patients and amniotic fluid from pregnant women.

Research has been conducted designed to detect specific IgE in patients suffering from toxoplasmosis. Known as an immunocapture assay, samples of CSF, fetal blood, umbilical cord blood, sera, and amniotic fluid were used. This technique is easy to perform and may prove to be helpful in diagnosing toxoplasmosis, particularly in pregnant women.

T. gondii tachyzoites, both invasive and intracellular, have been successfully demonstrated in AIDS patients suffering from pulmonary toxoplasmosis. A bronchoalveolar lavage was collected on each patient. Samples were then Giemsa-stained and microscopically examined.

Quick Quiz! 7-23

All the following are morphologic forms in the life cycle of *Toxoplasma gondii* except: (Objectives 7-5)

A. Oocysts
B. Tachyzoites
C. Bradyzoites
D. Sporozoites

Quick Quiz! 7-24

Human infection of *Toxoplasma* is initiated in all the following ways except: (Objectives 7-5)

A. Accidental ingestion of rodent feces
B. Ingestion of contaminated undercooked meat from cattle, pigs, or sheep
C. Transplacental infection
D. Transfusion of contaminated blood

Quick Quiz! 7-25

In which geographic area would you be likely to find *Toxoplasma gondii*? (Objective 7-2)

A. Tropics
B. Africa
C. United States
D. All of the above

Pneumocystis jiroveci (Pneumocystis carinii)

(new-moe"sis-tis/kah-reye"nee-eye)

Common associated disease and condition names: Pneumocystosis, atypical interstitial plasma cell pneumonia.

Morphology

Pneumocystis jiroveci, formerly called *Pneumocystis carinii*, is now considered as a fungus. However, its morphologic and biologic characteristics warrant inclusion in the discussion of miscellaneous protozoal parasites.

Trophozoites. The trophozoite or single organism, as it is often referred to, is the most commonly seen form (Fig. 7-14; Table 7-11). It

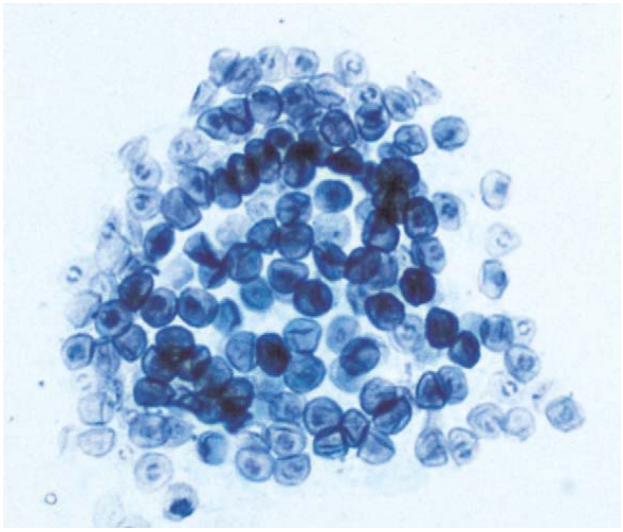

FIGURE 7-14 *Pneumocystis jiroveci,* multiple forms (iron hematoxylin stain, ×1000).

TABLE 7-11 ***Pneumocystis jiroveci* Trophozoite: Typical Characteristics at a Glance**

Parameter	Description
Size	2-4 μm
Shape	Ovoid, ameboid
Number of nuclei	One

TABLE 7-12 ***Pneumocystis jiroveci* Cyst: Typical Characteristics at a Glance**

Parameter	Description
Size	Diameter, 4-12 μm
Shape	Roundish
Number of nuclei	Four to eight; unorganized or arranged in a rosette

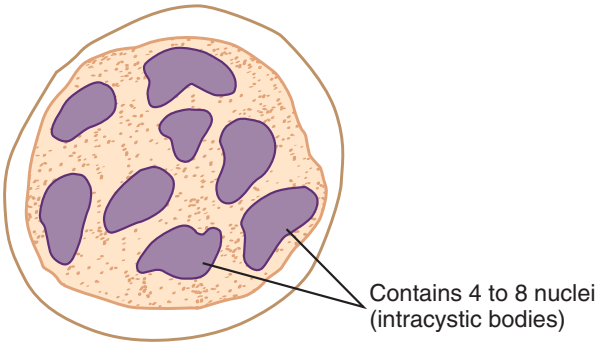

FIGURE 7-15 *Pneumocystis jiroveci* cyst.

is a simple ovoid and ameboid organism, measuring just 2 to 4 μm, with a single nucleus.

Cysts. The cysts of *P. jiroveci* contain four to eight intracystic bodies, also referred to in some sources as nuclei or trophozoites (Fig. 7-15; Table 7-12; see Fig. 7-14). These nuclei, as they will be called in this text, may be arranged in an organized fashion (in a rosette shape) or unorganized (scattered about the organism). The typical roundish cyst is relatively small, ranging in diameter from 4 to 12 μm.

Laboratory Diagnosis

Although Giemsa and iron hematoxylin stains may be used, successful diagnosis of *P. jiroveci* is usually done using histologic procedures, particularly Gomori's methenamine silver nitrate stain. Details of these histologic methods are beyond the scope of this text. Serologic techniques have been developed but are not yet considered to be appropriate for clinical diagnosis. Techniques such as the monoclonal immunofluorescent stain have also proven helpful in organism identification. Specimens that may be submitted for *P. jiroveci* examination vary and include sputum (usually obtained on individuals who are immunocompromised), bronchoalveolar lavage, tracheal aspirate, bronchial brushings, and lung tissue.

Life Cycle Notes

The life cycle of *P. jiroveci* is still considered as unknown. However, it has been presumed that once inside the host, *P. jiroveci* takes up residence in the alveolar spaces in lung tissue. Mature cysts rupture, producing actively growing, multiplying, and feeding trophozoites. The trophozoites eventually convert into precysts and cysts. The cycle would thus repeat itself. Sites other than lung have been known to harbor *P. jiroveci*, including the spleen, lung, lymph nodes, and bone marrow.

Epidemiology

P. jiroveci is prevalent in many parts of the world. Areas of particular note include the United States, Asia, and Europe. The route of organism transmission is believed to be via the transfer of pulmonary droplets through direct person-to-person contact. The population most at risk for contracting *P. jiroveci* is immunosuppressed patients, particularly those suffering from AIDS. Children, including malnourished infants and those with predisposing conditions such as a malignancy, have also been traditionally considered a high-risk group. *P. jiroveci*

has been known to pass through the placenta and to cause infection in the fetus as well as stillbirth.

Clinical Symptoms

Pneumocystosis: Atypical Interstitial Plasma Cell Pneumonia. In immunosuppressed adults and children, this condition results in a nonproductive cough, fever, rapid respirations, and cyanosis. These symptoms occur only a few days after onset. Interstitial plasma cell pneumonia is the leading cause of death in AIDS patients. It is interesting to note that AIDS persons infected with *P. jiroveci* often also suffer from Kaposi's sarcoma, a malignant skin disease. Infected malnourished infants experience poor feeding, loss of energy, a rapid respiration rate, and cyanosis. Onset is longer, lasting several weeks.

All infected patients typically exhibit an infiltrate on chest x-ray. Breathing difficulties may result in a low P_{O_2} (arterial oxygen tension) and a normal to low P_{CO_2} (carbon dioxide tension). Prognosis is usually poor. The lack of proper oxygen and carbon dioxide exchange in the lungs is the primary cause of death.

Treatment

Trimethoprim-sulfamethoxazole (Bactrim) is considered by many to be the first line treatment of infections caused by *P. jiroveci*. Pentamidine isethionate and cotrimoxazole are alternative treatments.

Prevention and Control

Because the life cycle of *Pneumocystis* is considered by some to be uncertain, prevention and control measures are obviously difficult to implement. However, based on the assumption that direct person-to-person contact through pulmonary droplets is the route of infection, personal protection from these droplets is crucial to prevent and control the spread of infection. Protective gear, such as a mask, worn around known infected persons may be one such measure.

Quick Quiz! 7-26

What is the preferred method of diagnosis for *Pneumocystis jiroveci*? (Objective 7-8)

A. Histologic stain
B. Giemsa stain
C. Iron hematoxylin stain
D. Iodine wet prep

Quick Quiz! 7-27

Which of the following groups of individuals is considered at highest risk for contracting *Pneumocystis jiroveci*? (Objective 7-6)

A. Veterans
B. Active military personnel
C. Immunosuppressed individuals
D. Newborns

Quick Quiz! 7-28

Pneumocystis jiroveci is believed to be spread via which of the following? (Objective 7-5)

A. Contaminated water
B. Mosquito bite
C. Person-to-person
D. Hand-to-mouth

LOOKING BACK

The miscellaneous protozoa described in this chapter have morphologic similarities (e.g., the oocysts of *Isospora* and *Sarcocystis*) and distinct differences (e.g., *Balantidium coli* versus *Blastocystis hominis*). When screening suspected samples, attention to organism size, shape, and structural details is imperative to identify parasites correctly. Organisms that are intestinal in nature, as well as the atrial protozoa, are