

The Intestinal Protozoa

A. Introduction

1. The Phylum Protozoa is classified into four major subdivisions according to the methods of locomotion and reproduction.
 - a. The amoebae (Superclass Sarcodina, Class Rhizopodea) move by means of pseudopodia and reproduce exclusively by asexual binary division.
 - b. The flagellates (Superclass Mastigophora, Class Zoomastigophorea) typically move by long, whiplike flagella and reproduce by binary fission.
 - c. The ciliates (Subphylum Ciliophora, Class Ciliata) are propelled by rows of cilia that beat with a synchronized wavelike motion.
 - d. The sporozoans (Subphylum Sporozoa) lack specialized organelles of motility but have a unique type of life cycle, alternating between sexual and asexual reproductive cycles (alternation of generations).
 - e. Number of species - there are about 45,000 protozoan species; around 8000 are parasitic, and around 25 species are important to humans.
2. Diagnosis - must learn to differentiate between the harmless and the medically important. This is most often based upon the morphology of respective organisms.
3. Transmission - mostly person-to-person, via fecal-oral route; fecally contaminated food or water important (organisms remain viable for around 30 days in cool moist environment with few bacteria; other means of transmission include sexual, insects, animals (zoonoses).

B. Structures

1. trophozoite - the motile vegetative stage; multiplies via binary fission; colonizes host.
2. cyst - the inactive, non-motile, infective stage; survives the environment due to the presence of a cyst wall.
3. nuclear structure - important in the identification of organisms and species differentiation.
4. diagnostic features
 - a. size - helpful in identifying organisms; must have calibrated objectives on the microscope in order to measure accurately.
 - b. type of motility - directional or non-directional; sluggish or fast.
 - c. cytoplasmic inclusions - chromatoid bars (coalesced RNA); red blood cells; food vacuoles containing bacteria, yeast, etc.
 - d. appearance of cytoplasm - smooth & clean or vacuolated.
5. endosome - also called the "karyosome," this is a mass of chromatin within

nucleus. The size, shape, and location of this structure are helpful in identification of organisms.

6. chromatin - nuclear DNA
7. chromatoid body or "bar" - coalesced RNA within the cytoplasm in the cyst stage. This is not always present, but when it is, its size and shape are helpful in determining species identification.

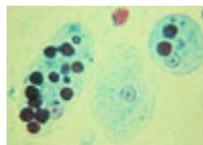
II. Class Lobosea - The Amoebae

A. Life cycle -

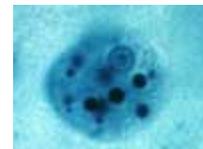
1. The definitive host ingests the infective cyst stage from fecal contamination in environment.
2. The cyst passes into the small intestine & excystation occurs with transformation to the trophozoite stage.
3. Trophozoites in the large intestine colonize the host by multiplying asexually via binary fission. They can remain in the lumen or invade the wall of the intestine (pathogenic species only) & multiply, from here they can be transported via the circulation to other organs (liver, lungs, etc.).
4. Cysts and trophozoites are passed in the feces of the infected host.
5. Infective stage - the mature cyst.
6. Diagnostic stage - the trophozoite or cyst in stool or tissue specimens.

B. Genus *Entamoeba* - contains the most important of the amoebae causing disease in humans.

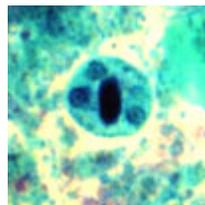
1. *Entamoeba histolytica*



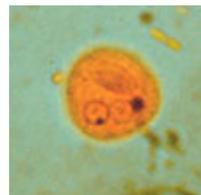
Entamoeba histolytica trophozoites with ingested red blood cells



Entamoeba histolytica trophozoite with ingested red blood cells



Entamoeba histolytica cyst with chromatoid bar



Entamoeba histolytica cyst with chromatoid bar (iodine stain)



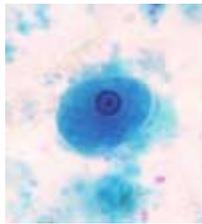
E. histolytica cyst

- a. Epidemiology - Occurs worldwide; the highest incidence and prevalence is found in areas with poor sanitation where as many as 80% of a population may be infected. Highest in children >5 years of age; more

prevalent in males than in females; common in mental hospitals, prisons, orphanages.

- b. Pathology and Clinical Manifestations - the most pathogenic of all; causes amoebic dysentery; can become extra-intestinal (liver, lungs, etc.); can be fatal.
- c. Morphology & Laboratory Identification - the trophozoite ranges between 12 and 30 microns in diameter. Its nucleus has an even distribution of peripheral chromatin and a small, compact, centrally located karyosome. The cytoplasm is typically smooth and granular with inclusions, if present, containing red blood cells. The cyst ranges between 10 and 30 microns in diameter and contains four nuclei when mature. Cigar-shaped chromatoid bars may be present in some cysts.
- d. Treatment - depends upon location of infection (lumen vs. tissue)
- e. Distribution - worldwide, mostly in tropics and sub-tropics.
- f. Note - chronic infections may last for years; they are often confused with other conditions (colitis, cancer). Hepatic abscess is the most common and dangerous complication.

2. *Entamoeba hartmanni*



Entamoeba hartmanni trophozoite
trichrome stain



Entamoeba hartmanni trophozoite
iron-hematoxylin stain



Entamoeba hartmanni cyst (iodine stain)

- a. Formerly called the “small race” of *Entamoeba histolytica*.
- b. Technologists must be able to differentiate this organism from *E. histolytica* because *E. hartmanni* is non-pathogenic.
- c. Morphology & Laboratory Identification - this organism is very similar to *E. histolytica*. The difference lies in the respective sizes of the organisms. Trophozoites of *Entamoeba hartmanni* will measure less than 12 microns, while cysts will measure less than 10 microns. It is strongly recommended that more than one organism be measured when determining size.

2. *Entamoeba coli*



Entamoeba coli trophozoite



E. coli trophozoite



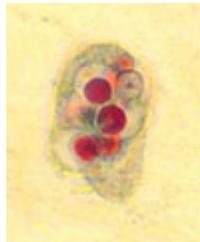
E. coli cyst



E. coli cyst (iodine)

- a. Morphology - trophozoites range from 10 to 35 microns in diameter; cysts range from 10 to 30 microns in diameter and contain 8 to 16 nuclei when mature; the nucleus exhibits an eccentric karyosome with irregular, coarse chromatin. The cytoplasm is heavily vacuolated, containing yeast, bacteria, and debris.
- b. Significance - this is a harmless commensal but must be differentiated from the pathogens.

2. *Entamoeba gingivalis*

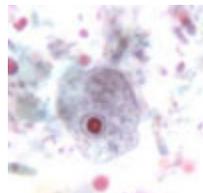


Entamoeba gingivalis trophozoite

- a. Infective site - in the mouth; the organism thrives in diseased gums, but is not considered a causal agent or pathogen. If swallowed, it is destroyed in stomach.
- b. Transmission - contact with fomites (drinking glasses, eating utensils, etc.; kissing).
- c. Morphology - resembles *E. histolytica*, but has no cyst stage. It is the only species, which ingests leucocytes.

C. Other amoebae of significance

1. *Endolimax nana*



Endolimax nana trophozoite



E. nana cyst

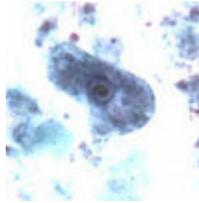


E. nana cyst (iodine)

- a. Occurrence - occurs in about 14% of the US population; 21% worldwide.

- b. Pathogenicity - none.
- c. Morphology - trophozoites range from 5 to 10 microns in diameter. The nucleus contains a large, blot-like karyosome and has little or no peripheral chromatin. Cysts are usually sub-oval, measuring 4 to 6 by 6 to 10 microns

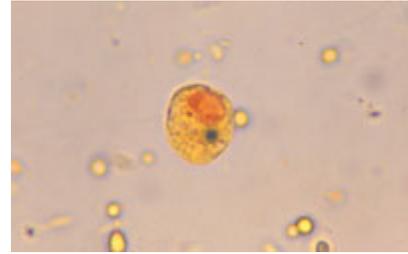
2. *Iodamoeba butschlii*



Iodamoeba butschlii trophozoite



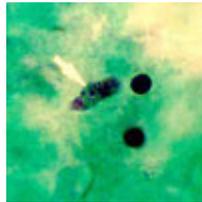
I. Butschlii cyst



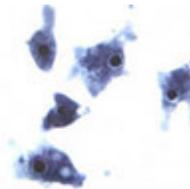
I. Butschlii cyst (iodine)

- a. Pathogenicity - none.
- b. Morphology – often called iodine cyst - striking characteristic is large glycogen vacuole - stains dark brown with iodine.

3. *Naegleria fowleri*



Naegleria fowleri trophozoite in CSF



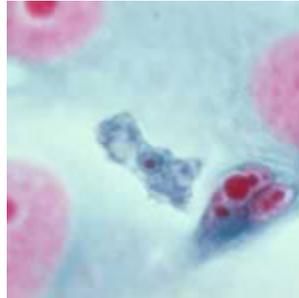
N. fowleri trophozoites

- a. Classification - an ameboflagellate and a free-living organism; it alternates between amoeboid and bi-flagellated forms; only the amoeboid form is found in tissues. Disease occurs during summer months. Occurs only in fresh water, can tolerate chlorinated swimming pools.
- b. Life cycle - the amoeba gains entry via the nasal mucosa, usually during a swimming event; it moves along the olfactory nerve, gaining access to the brain via the cribriform plate. It rapidly colonizes the brain, and cases are invariably fatal. Infections do not spread from person-to-person.
- c. Symptoms - Symptoms are dramatic and rapidly progressive. Headache, fever, nausea & vomiting occur within 1 to 2 days. Meningoencephalitis, irrational behavior, coma & death usually occur within 9 days of exposure.
- d. Diagnosis - Usually made at autopsy. CSF contains a large number of segmenters with no bacteria present. Motile amoebae can be seen in wet mounts of CSF. Early diagnosis is critical. CSF specimens can be plated onto non-nutrient agar containing a lawn of bacteria; amoebae, which grow out, can be transferred into sterile filtered water to determine

if the organisms present transform into the bi-flagellated stage.

- e. Treatment - Amphotericin B (Intravenous and Intrathecal) with rifampin is the only regimen to date that has proven successful in saving a patient.

4. *Acanthamoeba* spp.



Acanthamoeba spp. trophozoite

- a. Life cycle - also a free-living amoeba. The amoeba reaches the brain hematogenously after entering a wound or lesion on the skin. More commonly, the organism is associated with getting into eyes via contaminated or homemade cleaning solutions.
- b. Symptoms - slow onset (10 or more days). Presents as chronic, granulomatous lesions in brain. In eye lesions, the infection resembles a herpes virus infection.
- c. Acanthamoeba keratitis - affects healthy persons; sometimes associated with eye trauma; most recently has been associated with users of extended-wear contact lenses. Cysts are resistant to drying and disinfection via chlorine.

III. Superclass Mastigophora - the flagellates; members of this group can inhabit mouth, bloodstream, tissues, gastrointestinal, or urogenital tracts.

A. Members:

Giardia lamblia
Dientamoeba fragilis
Chilomastix mesnili
Trichomonas hominis
*Retortamonas intestinalis**
*Enteromonas hominis**

* will not be discussed- nonpathogenic, very rare, and of no medical significance.

B. Morphological Characteristics

- 1. Flagellum(ae) - characteristic organelle of locomotion. It is an extension of ectoplasm and resembles a tail; moves with a whip-like motion.
- 2. Axostyle - a supporting mechanism; a rod-shaped structure; not all Genera exhibit these.
- 3. Undulating membrane - a protoplasmic membrane with a flagellar rim extending

out like a fin along the outer edge of the body of some flagellates. Moves in a wave-like motion.

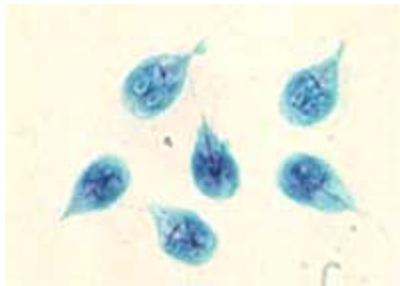
4. Costa - a thin, firm rod-like structure running along the base of the undulating membrane in some flagellates.
5. Cytosome - a rudimentary mouth; also referred to as a gullet.

C. Identification of a flagellate is based upon:

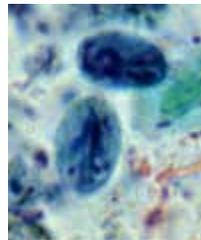
1. Size
2. Shape
3. Motility
4. Number and morphology of nuclei
5. Number and location of flagellae
6. Location in the body of the host

D. Intestinal flagellates

1. *Giardia lamblia* - probably the first described protozoan pathogen of humans.



Giardia lamblia trophozoites



G. lamblia cysts

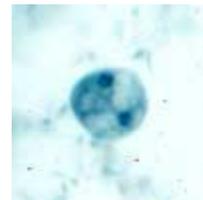
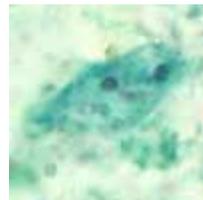


G. lamblia cyst (iodine)

- a. Most common protozoan parasite in the U.S.A.
- b. Life cycle - man ingests cysts from fecally contaminated environment; the organism excysts in the upper intestine; trophozoites multiply and attach to the intestinal mucosa, sometimes entering secretory tubes, even the gall bladder. Trophozoites and cysts are passed in the feces.
- c. diagnosis - identification of cysts or trophozoites in stool specimens or duodenal contents. Irregular shedding pattern results in a "showering" of organisms at times, while being difficult to detect at other times.
- d. Morphology - very distinctive. Dorsal-ventrally flattened, and Bi-laterally symmetrical.
 - 1) Cyst - Measures 9 x 12 micrometers and contain 2 to 4 nuclei; the karyosome is centrally located, with little or no peripheral chromatin; parabasal bodies are present.
 - 2) Trophozoite - Four pairs of flagella - one pair located anterior, two pair located ventral, and one pair located posterior. An axostyle and parabasal bodies are present. motility resembles a "falling leaf" uses "sucking discs" to adhere to intestinal wall; interferes with absorption of nutrients

- e. Epidemiology - prevalence 1 to 30%, depending upon the population surveyed; often occurs in epidemics, especially in children's day care centers; can be transmitted in water. Cysts remain viable as long as 3 months when protected from direct sunlight and excess heat; resistant to chlorination. Sexual transmission has been well documented.
- f. Pathology and Clinical Manifestations - symptoms can be severe; diarrhea, foul - smelling, greasy, mucus-laden stools, flatulence, nausea, cramps. Most infections are asymptomatic; chronic cases experience weight loss, malabsorption of fat, protein, folic acid, and fat-soluble vitamins.

2. *Dientamoeba fragilis*



Dientamoeba fragilis trophozoite *D. fragilis* trophozoite *D. fragilis* trophozoite

- a. General - Until recently, classified as an amoeba; electron microscopy and immunological studies have recently suggested a flagellate nature.
- b. Laboratory diagnosis - detection of bi-nucleated (40% - 60%) trophozoites with fragmented karyosomes, each karyosome consisting of 4 to 8 granules of chromatin.
- c. Diagnostic stage - the trophozoite in feces. There is no cyst stage known.
- d. Morphology
 - 1) 1 to 2 nuclei, with little or no peripheral chromatin
 - 2) karyosome is divided into 4 to 8 distinct granules
 - 3) may rarely ingest cells
- e. Pathology and Clinical Manifestations - infection is usually asymptomatic; can be associated with diarrhea, anorexia, abdominal pain.
- f. Distribution - worldwide, 1 - 20% prevalence.
- g. Association with the pinworm *Enterobius vermicularis* - it is thought that the organism is transmitted from host to host within the egg of this nematode.

2. *Chilomastix mesnili*



Chilomastix mesnili trophozoite



C. mesnili cyst

- a. A non-pathogen - must be differentiated from Giardia.
- b. A commensal organism
- c. Found in cecum and colon
- d. Transmission - by ingestion of mature cysts.
- e. Morphology
- f. trophozoite
 - 1) 4 flagella (3 extend anteriorly, and 1 is associated with the cytostome); provide jerky directional movement
 - 2) curved posterior
 - 3) single nucleus - small eccentric karyosome, with granular nuclear chromatin; the nucleus is always located anteriorly.
- g. cyst
 - 1) distinctive lemon shape
 - 2) thick-walled with clear knob at one end
 - 3) evidence of cytostome may be seen
 - 4) 1 nucleus

4. The Trichomonads

- a. Characteristics:
 - 1) Undulating membrane - protoplasmic membrane with flagellar rim extending out like a fin along outer edge of body. Moves in a wave-like fashion.
 - 2) Flagella - several in a tuft, provides locomotion
 - 3) Axostyle - functions for support
 - 4) Costa - firm rod-like structure running along base of the undulating membrane.
 - 5) Cytostome - rudimentary mouth

b. *Trichomonas hominis* – a non-pathogen



Trichomonas hominis trophozoite

- 1) Commensal - must differentiate from pathogens
- 2) Transmission - direct person-to-person fecal transmission.
- 3) No cyst stage
- 4) Must differentiate from *T. vaginalis* - in instances where feces is contaminated with urine.
- 5) Morphology - “arc-shaped” exhibiting a wobbly, jerky, yet progressive motility

b. *Trichomonas vaginalis*



Trichomonas vaginalis trophozoites

- 1) Life cycle - trophozoite lives in the vagina, urethra, epididymis, and prostate; multiplies via longitudinal fission; no cyst stage.
- 2) Mode of infection - sexual or fomites.
- 3) Diagnosis - ID of trophs in body fluids - wet mounts of discharges or on PAP smears.
- 4) Pathology and Clinical Manifestations - females: vaginal discharge; burning, itching, or chafing. Frequency of urination or dysuria. Relationship between trichomoniasis & cervical cancer. Males: frequently asymptomatic. If the prostate is involved, the patient may develop discharge, dysuria, and enlargement of prostate with tenderness. There are reports of infections (acquired during vaginal birth) of upper respiratory tract and conjunctiva of newborns from infected mothers.
- 5) Morphology - exists only in trophozoite stage has an axostyle and short undulating membrane that extends less than half the body length; exhibits 4 flagellae, arranged in a tuft

IV. Class Kinetofragminophora - The Ciliate

A. *Balantidium coli*



Balantidium coli trophozoite *Balantidium coli* trophozoite (unstained)

1. Epidemiology

Rarely found in USA. This is the only ciliate parasite of humans. It is prevalent in tropical areas, or where poor sanitation, hygiene, and crowding occur. Increase numbers of infections are expected in those with close, continuing contact with swine.

2. Largest parasitic protozoan - the trophozoite is 30-120 x 25-125 microns; the cyst averages 50 - 70 microns in diameter.

3. Pathology & Symptoms

- a. Many infections are asymptomatic, with organism feeding on bacteria at surface of mucosa.
- b. Severe intestinal infections - with aid of hyaluronidase secretions, organism burrows into submucosa, producing ulcers like those of *E. histolytica*. Symptoms include dysentery, abdominal pain, nausea & vomiting, fever, headache.

4. Life cycle - similar to that of *E. histolytica*.

- a. The cyst is ingested via person-to-person transmission, or fecal contamination in environment.
- b. The cyst excysts in the small intestine
- c. Trophozoites migrate to large intestine

5. Diagnosis

Diagnosed by observing cysts & trophs in fecal samples. Cysts are easily missed – cysts stain very dark with iodine, so the structures used in identification (buccal cavity & macronucleus) are not always readily visible.

6. Morphology

- a. Large, ovoid shape.
- b. Contains two nuclei, 1 large kidney shaped (macronucleus) & 1 small subspherical micronucleus adjacent to the macronucleus. Micronucleus is difficult to see, if seen at all.

- c. Body surface covered by spiral, longitudinal rows of cilia that provide rotary, boring motility.
7. Disease names
 - a. balantidiasis
 - b. balantidial dysentery
 8. Primary animal reservoir - pigs; monkeys probably of secondary importance.

V. Intestinal Coccidia

A. Introduction

1. A number of species parasitize humans: *Isospora*, *Sarcocystis*, *Cryptosporidium*, & *Toxoplasma*
2. Have complex life cycles - most have 2-host life cycle.
3. Schizogony - asexual binary fission.
4. Sporogony -sexual reproduction
5. Diagnostic stages are often difficult to locate. They are easily overlooked due to their nearly transparent appearance. Permanently stained smears not helpful. Acid fast and giemsa stains are more often used. Oocysts do not stain with iodine.

B. *Sarcocystis*



Sarcocystis spp. oocysts

1. Pathology - *Sarcocystis bovihominis* & *S. suihominis* are intestinal infections similar to *Isospora belli*. *Sarcocystis lindemanni* is the name given to the organism, which causes infection of the muscle.
2. Definitive host - humans. Pig (sui-) and cow (bovi-) are intermediate hosts.
3. Infective stage - ingestion of sarcocysts in meat (intestinal); ingestion of oocysts from animal feces (muscle).

B. *Isospora belli*



Isospora belli oocyst (mature) *Isospora belli* oocyst (immature)

1. Definitive host - humans.
2. Schizogony and sporogony:

Schizogony - takes place upon initial infection when sporozoites escaping from ingested oocysts invade the intestinal epithelium and multiply. The resulting schizont releases trophozoites which invade other epithelial cells, and so the infection progresses. Sporogony - sexual reproduction in which some of the progeny of asexual reproduction initiate development into gametes (male & female); microgametes migrate into lumen of bowel, and fertilize macrogametes within epithelial cells; fertilized macrogametes develop into oocysts which are passed as the infectious stage in the feces.

3. Diagnostic/infective stage
 - a. Immature oocysts - contain only one sporocyst, do not stain with iodine; measure 12 x 30 microns.
 - b. Mature oocysts - contain two sporocysts, each of which contain four sporozoites. Similarly, do not stain with iodine.
 - c. The sporozoites are released when the oocyst wall is digested away in the small intestine, they then invade the epithelial cells.
 - d. They develop into trophozoites that multiply by binary fission (schizogony) and are released when the infected epithelial cell ruptures.
 - e. This process can be repeated, or the organisms can undergo a transformation into macrogametocytes & microgametocytes and initiate a cycle of sexual reproduction.
4. Intermediate hosts - none.
5. Pathology - ranges from asymptomatic to acute, severe dysentery.

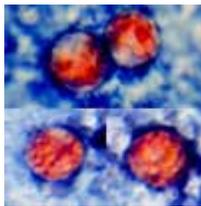
C. *Cryptosporidium parvum*



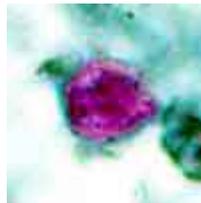
Cryptosporidium parvum oocysts
(acid-fast stain)

1. Site of infection - primarily an intestinal infection, cryptosporidiosis can become systemic in AIDS patients. Organisms develop within the microvillous region of epithelial cells lining the small intestine.
2. Originally considered an animal parasite (rodents, cattle and sheep), it is now known that a wide variety of animals are naturally infected. Organisms from some (fish, fowl and reptiles) are not believed to be able to infect humans.
3. Transmission - an endemic cycle is maintained via person-to-person, fecal-oral route transmission. The parasite is a relatively common finding in "day-care" diarrhea. It can be sexually transmitted. It exhibits a marked potential for being waterborne due to significant resistance to disinfection. The potential for animal-to-person transmission should not be underestimated.
4. Infections occur primarily in people with compromised immune systems. These can be serious infections. It is readily accepted that the organism will readily infect immunocompetent persons also. It is rapidly becoming a pediatric disease.
5. Pathology - although the condition can be asymptomatic, most infections cause moderate to severe diarrhea of one to two weeks in the immunocompetent patient. In the immunosuppressed patient, the condition is protracted and life threatening. At this time, there is no drug effective against this parasite.
6. Identification - oocysts are 2 - 5 microns in diameter; do not stain with iodine; and are acid-fast.

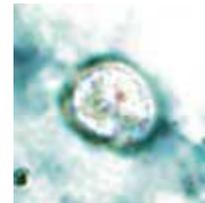
D. *Cyclospora cayetanensis*



Cyclospora cayetanensis oocysts
(acid-fast stain)



Cyclospora cayetanensis oocyst
(acid fast stain)



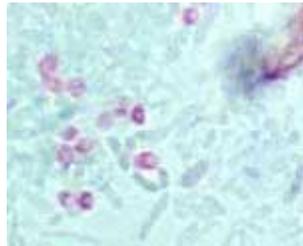
Cyclospora cayetanensis oocyst
(Variable acid fast reaction)

1. General - Relatively new to this country, this parasite has been detected in the stools of infected individuals in the tropics for quite some time. The first outbreak in the USA occurred in medical residents in Chicago in 1980, and a large multi-state outbreak occurred the summer of 1996. During this outbreak, Texas had more than 100 cases over a period of about six weeks, while no more than one case had been reported during any previous year.
2. Animal reservoirs - not known. Other *Cyclospora* species are known to infect a variety of animals, but *C. cayetanensis* is the name designated for the only one known to infect humans (at this time).
3. Transmission - Contaminated food or water. The Chicago outbreak was epidemiologically associated with drinking water; the summer 1996 outbreak was tied to contaminated raspberries (imported from Central America) and possibly to strawberries grown in California. Due to an extended time period (~10 - 14 days)

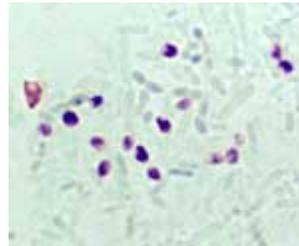
being required for oocyst sporulation, person-to-person spread is considered unlikely.

4. Pathology - Infected individuals experience a diarrhea similar to that experienced with *Cryptosporidium* infections. While some cases were less debilitating than others, asymptomatic cases were not thought to have occurred. Trimethoprim-sulfamethoxazole is an effective treatment in "non-sulfa sensitive" individuals.
5. Identification - Oocysts are spherical, 7 - 10 microns in diameter, and present as variably acid-fast. Acid-fast procedures utilizing carbol-basic fuchsin proved superior in demonstrating this parasite.

E. Microsporidium



Microsporidium spp. spores



Microsporidium spp. spores

1. General – as of this time, infections are thought to be limited to AIDS patients.
2. Transmission – person-to-person, via the fecal-oral route; can be sexually transmitted during sex practices involving contact with feces.
3. Pathology - similar to that of *Cryptosporidium* infections.
4. Identification – a very small organism, spores average 1.0 by 1.7 microns. They stain a reddish-pink color with Chromotrope stain. Some stained spores exhibit a dark staining "belt" across the middle of the organism.