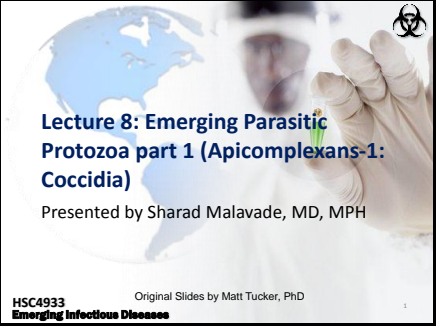
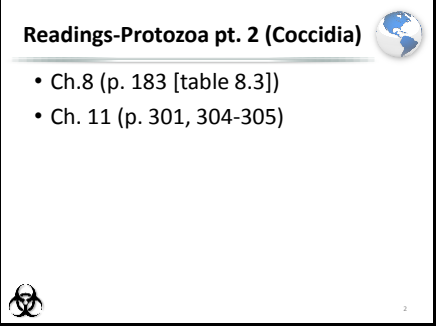




<p>Slide 1</p>		<p>This lecture is the second part of the protozoal parasites. In this LECTURE we will talk about the Apicomplexans SPECIFICALLY THE Coccidians. In the next lecture we will talk about the Plasmodia and Babesia</p>
<p>Slide 2</p>		<p>These are the readings for this week.</p>
<p>Slide 3</p>		

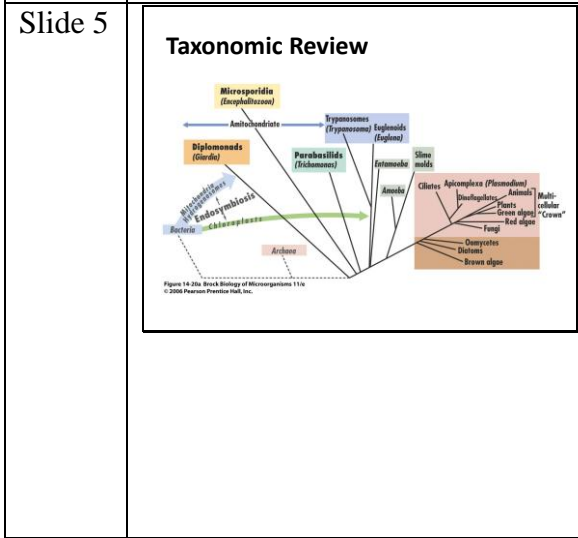
**Slide 4**

**Learning objectives: Apicomplexan coccidia**

- Define basic attributes of Apicomplexans- unique characteristics?
- Know basic life cycle and developmental stages of coccidian parasites
- Required hosts
  - Transmission strategy
  - Infective and diagnostic stages
  - Unique character of reproduction
- Know the common characteristics of each parasite
  - Be able to contrast and compare
- Define diseases, high-risk groups
- Determine diagnostic methods, treatment
- Know important parasite survival strategies
- Be familiar with outbreaks caused by coccidians and the conditions involved



These are the learning objectives for this lecture.

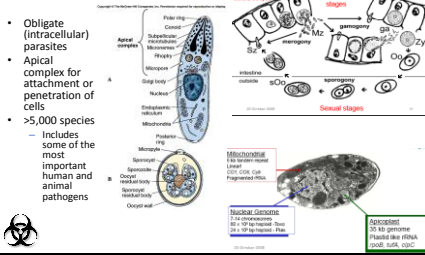
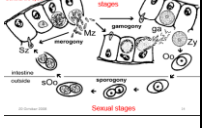
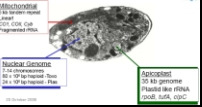


This figure from the last lecture is just to show you the apicomplexans. This lecture we talk about the Coccidians.

**Slide 6**

**Phylum Apicomplexa (the Sporozoa)**

- Obligate (intracellular) parasites
- Apical complex for attachment or penetration of cells
- >5,000 species
  - Includes some of the most important human and animal pathogens

**Apical Complex:**  
2-16 sporozoites  
2-4 merozoites  
8-16 oocysts  
8-16 sporozoites

**Sexual stages:**  
Macrogamete  
Microgamete

**Nuclear Genomes:**  
2-16 sporozoites  
2-4 merozoites  
8-16 oocysts  
8-16 sporozoites

**Apicomplexan Mitochondrion:**  
2-16 sporozoites  
2-4 merozoites  
8-16 oocysts  
8-16 sporozoites

**Apicomplexan Chloroplast:**  
2-16 sporozoites  
2-4 merozoites  
8-16 oocysts  
8-16 sporozoites

The phylum Apicomplexa is so named because of the complex of subcellular organelles that are aggregated at the anterior end of the organism and that help in the penetration in to the host cell. This phylum has over 5000 species of organisms and most are pathogenic to humans, animals or both. The apicomplexans are obligate intracellular parasites. The second figure on the top right shows the generic life-cycle of the apicomplexans- both the sexual and asexual. The bottom right image shows the three regions that bear genomic material in the apicomplexans.

Slide 7

**Lecture 8: On the Menu-Coccidia**



**Phylum Apicomplexa, Class Conoidasida**

- *Isospora belli*
- *Cryptosporidium* spp.
- *Cyclospora cayetanensis*
- *Toxoplasma gondii*



In this lecture we will talk about the apicomplexans belonging to sub-class Coccidia: *Isospora belli*, *Cryptosporidium parvum*, *Cyclospora cayetanensis* and *Toxoplasma gondii*.

Slide 8

**Coccidia**



- Spore-forming, single-celled parasites belonging to the apicomplexan class Conoidasida, subclass Coccidiasina
- Infect the intestinal tracts, liver, kidneys, blood cells, other tissues of vertebrates and invertebrates
- Largest group of apicomplexan protozoa
- Characterized by a thick walled **oocyst stage** that is typically excreted with the feces



Coccidia are single spore forming parasites. They infect the intestinal tract, liver, kidneys, blood cells and other tissues of both vertebrates and invertebrates. They are the largest group of apicomplexan protozoa and are characterized by a thick walled oocyst stage that is excreted in the feces.



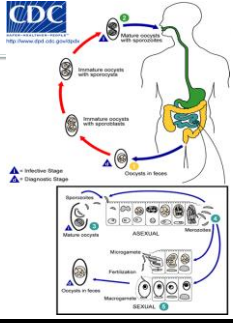

Slide 9

**TABLE 18.3 Opportunistic Infections Associated With AIDS**

Microbe	Disease	Characteristics
<b>Bacteria</b>		
<i>Mycobacterium tuberculosis</i>	Tuberculosis	Primarily lungs, but dissemination possible
<i>Mycobacterium avium</i>	Extrapulmonary tuberculosis	Lungs and other organs
<i>Mycobacterium intracellulare</i>	Disseminated infection in immunocompromised persons	
<i>Salmonella</i> sp.	Salmonellosis	Gastrointestinal symptoms
<i>Legionella pneumophila</i>	Legionnaires disease	Pneumonia-like respiratory symptoms
<b>Viruses</b>		
Herpes simplex virus	Herpes	Lesions on skin and mucous membranes
Cytomegalovirus	Cytomegalovirus	Encephalitis, blindness
<i>Vaccinia zoster</i> virus	Shingles	Painful, itchy lesions on skin
Epstein-Barr virus	Heavy leukoplakia	Lesions in mouth
<b>Protozoans</b>		
<i>Pneumocystis jirovecii</i>	Pneumonia	Pneumonia-like respiratory symptoms
<i>Cryptosporidium parvum</i>	Cryptosporidiosis	Diarrhea
<i>Toxoplasma gondii</i>	Toxoplasmosis	Brain and nervous system
<i>Isospora belli</i>	Isosporiasis	Diarrhea
<b>Fungi</b>		
<i>Candida albicans</i>	Candidiasis	Lesions on throat, pharynx, lungs
<i>Cryptococcus neoformans</i>	Cryptococcosis	Meningitis and other disseminated symptoms
<i>Histoplasma capsulatum</i>	Histoplasmosis	Pneumonia and other disseminated symptoms

Like Microsporidia, these other protozoan parasites are problems for immunocompromised individuals

This table lists the major opportunistic infections in patients with AIDS. Of our interest here for the purpose of this lecture are *Cryptosporidium parvum*, *Isospora belli* and *Toxoplasma gondii*. Note the disease characteristics associated with these infections.

<p>Slide 10</p>	<p><b><i>Isospora belli</i></b></p>  <ul style="list-style-type: none"> <li>• Only infects man</li> <li>• Least common of the three intestinal coccidia that infect humans.</li> <li>• Infects the epithelial cells of the small intestine</li> <li>• Found worldwide, especially in tropical and subtropical areas.</li> <li>• <b>Infection occurs in immunosuppressed individuals, and outbreaks have been reported in institutionalized groups in the United States</b></li> <li>• U.S.-Infections are more commonly observed in Hispanics, foreign-born patients, and HIV-positive homosexual men rather than those who acquired HIV by some other route such as intravenous drug use</li> </ul> 	<p><i>Isospora belli</i>, is a coccidian parasite that infects man. Some others infect dogs. In fact it is the least common of the coccidian parasites to cause infection. The organism infects the intestinal epithelial cells. It has a global distribution. In some tropical and sub-tropical countries, 20% AIDS patients with diarrhea have isospora. In the US it is most commonly seen in Hispanic individuals, foreign born patients, HIV positive homosexual men rather than those who acquire HIV by some other route such as IV drug use.</p>
<p>Slide 11</p>	<p><b>Isosporiasis</b></p>  <p>Note large oocyst with two sporocysts inside (these in turn contain sporozoites)</p> <p>This part of parasite development is similar for other coccidia</p> 	<p>Infection occurs by ingestion of sporocysts-containing oocysts: the sporocysts excyst in the small intestine and release their sporozoites, which invade the epithelial cells and initiate schizogony. Upon rupture of the schizonts, the merozoites are released, invade new epithelial cells, and continue the cycle of asexual multiplication. The merozoites upon invasion of a new epithelial cell form trophozoites that develop into schizonts which contain multiple merozoites. After a minimum of one week, the sexual stage begins with some of the trophozoites leading to development of male and female gametocytes. Fertilization results in the development of oocysts that are excreted in the stool.</p> <p>At time of excretion, the immature oocyst contains usually one sporoblast (more rarely two). In further maturation after excretion, the sporoblast divides in two (the oocyst now contains two sporoblasts); the sporoblasts secrete a cyst wall, thus becoming sporocysts; and the sporocysts divide twice to produce four sporozoites each.</p>

Slide

12

### Isosporiasis

- Disease**- Acute nonbloody diarrhea with crampy abdominal pain, which can last for weeks and result in malabsorption and weight loss.
  - In immunodepressed patients, and in infants and children, the diarrhea can be severe.
- Diagnosis**-ID of large, typically shaped oocysts in stool, found in biopsies
  - Differential interference contrast (DIC), and epifluorescence.
  - PCR of stool samples can be used for diagnosis as well (blue arrow)
- Treatment**- Trimethoprim-sulphamethoxazole
- Prevention**-eliminate fecal-oral transmission, improved personal hygiene, sanitation

Isospora is usually a rare disease in humans. It can cause problems in certain groups of patients like those with HIV infection. The diarrhoea is associated with abdominal cramps and can last for a prolonged period and results in malabsorption. It can be very severe in individuals with severe immunocompromise. The diagnosis is made by microscopic examination of stool samples that reveal the large characteristic oocysts. Treatment is with Trimethoprim Sulfamethoxazole. Prevention is primarily by elimination of fecal oral transmission by improved personal hygiene and sanitation.

Slide

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### An emerging threat?

- Unlike crypto and cyclo, doesn't cause large outbreaks

Frequency of Emerging Parasites in HIV/AIDS and Oncological Patients Stool by Cryptological and Molecular Analysis

Figure 1. Frequency of parasites identified in stool from HIV/AIDS (n=100) and oncological patients (n=100). Quantification (Number per gram) and stool volume (mL).

Frequency of Emerging Parasites in HIV/AIDS and Oncological Patients Stool by Cryptological and Molecular Analysis. Mendes-Jones-Carvalho et al. Advances in Parasitology, 2015, 8, 169-179. doi:10.1016/j.apar.2015.05.005. Parasites Online September 2015. <http://www.sci.org/journal>

Unlike cryptosporidium and cyclospora, Isospora does not cause large outbreaks. This graphic from a recent paper shows the small number of Isospora belli infections compared to crypto and cyclospora in immunocompromised individuals.

Slide

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### Can be a chronic problem in AIDS patients

#### Drug resistance

Case number	1	2	3	4	5	6	7	8
Gender	M	F	M	M	F	M	F	M
Age (years)	32	35	41	32	32	35	34	31
CD4 count (cells/mm <sup>3</sup> )	100	100	100	100	100	100	100	100
Time since diagnosis (years)	10	10	10	10	10	10	10	10
Time since diagnosis (months)	12	12	12	12	12	12	12	12
Time since diagnosis (weeks)	12	12	12	12	12	12	12	12
Time since diagnosis (days)	12	12	12	12	12	12	12	12
Time since diagnosis (hours)	12	12	12	12	12	12	12	12
Time since diagnosis (minutes)	12	12	12	12	12	12	12	12
Time since diagnosis (seconds)	12	12	12	12	12	12	12	12
Time since diagnosis (milliseconds)	12	12	12	12	12	12	12	12
Time since diagnosis (microseconds)	12	12	12	12	12	12	12	12
Time since diagnosis (nanoseconds)	12	12	12	12	12	12	12	12
Time since diagnosis (picoseconds)	12	12	12	12	12	12	12	12
Time since diagnosis (femtoseconds)	12	12	12	12	12	12	12	12
Time since diagnosis (attoseconds)	12	12	12	12	12	12	12	12
Time since diagnosis (zeptoseconds)	12	12	12	12	12	12	12	12
Time since diagnosis (yoctoseconds)	12	12	12	12	12	12	12	12

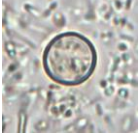

Boyles TH, Black J, Manríquez G, Mendelson M (2012) Failure to Eradicate Isospora belli Diarrhea Despite Immune Reconstruction in Adults with HIV - A Case Series. PLOS ONE 7(8): e42844. doi:10.1371/journal.pone.0042844  
<http://www.plosone.org/pdoi/10.1371/journal.pone.0042844>

However, it can be a problem in HIV patients. There have been some reports of drug resistance. This table from a recent paper shows the case descriptions of 8 patients who had recurrent diarrhoea due to Isospora belli despite therapy.

Slide  
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**Cyclospora cayetanensis**

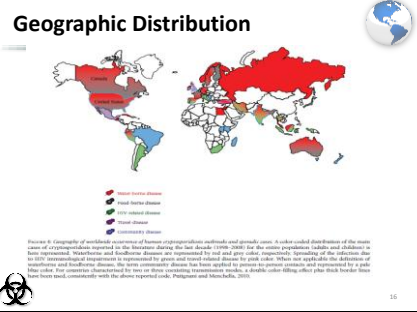

- The first human cases of were reported in 1979
- The species designation *Cyclospora cayetanensis* was given in 1994 to Peruvian isolates of human-associated *Cyclospora*.
- Not immediately infective
- 1100 lab confirmed cases reported to CDC from 1997-2008

*Cyclospora cayetanensis* is another type of coccidian parasite that you more likely to be familiar with. The first reported cases were from 1979. The species designation was given in 1994 to the Peruvian isolates of human-associated *Cyclospora*. The parasite needs some time to mature and become infective and so it is not infective immediately as in some other parasites. 1,110 laboratory-confirmed sporadic cases of cyclosporiasis were reported to the CDC from 1997 to 2008. The overall population-adjusted incidence rates ranged from a low of 0.01 cases per 100,000 persons in 1997 to a high of 0.07 in 2002.

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**Geographic Distribution**

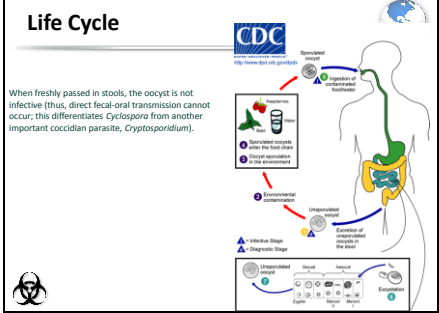




Cyclosporiasis has been reported worldwide, but is most common in Latin America, the Indian subcontinent, and southeast Asia. The graphic from a 2010 review article shows the color coded mode of transmission.

Slide  
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**Life Cycle**

When freshly passed in stools, the oocyst is not infective (thus, direct fecal-oral transmission cannot occur; this differentiates *Cyclospora* from another important coccidian parasite, *Cryptosporidium*).

Let's look at the lifecycle of cyclospora now. When freshly passed in stools, the oocyst is not infective (thus, direct fecal-oral transmission cannot occur; this differentiates *Cyclospora* from another important coccidian parasite, *Cryptosporidium*). In the environment, sporulation occurs after days or weeks at temperatures between 22°C to 32°C, resulting in division of the sporont into two sporocysts, each containing two elongate sporozoites. Fresh produce and water can serve as vehicles for transmission and the sporulated oocysts are ingested (in contaminated food or water). The oocysts excyst in the gastrointestinal tract, freeing the sporozoites which invade the epithelial cells of the small intestine. Inside the cells they undergo asexual multiplication and sexual development to mature into oocysts, which will be shed in stools.

When freshly passed in stools, the oocyst is not infective (thus, direct fecal-oral transmission cannot occur; this differentiates *Cyclospora* from another important coccidian parasite, *Cryptosporidium*). In the environment, sporulation occurs after days or weeks at temperatures between 22°C to 32°C, resulting in division of the sporont into two sporocysts, each containing two elongate sporozoites. Fresh produce and water can serve as vehicles for transmission and the sporulated oocysts are ingested (in contaminated food or water). The oocysts excyst in the gastrointestinal tract, freeing the sporozoites which invade the epithelial cells of the small intestine. Inside the cells they undergo asexual multiplication and sexual development to mature into oocysts, which will be shed in stools. The potential mechanisms of contamination of food and water are still under investigation.

Slide  
18

**Clinical Features**



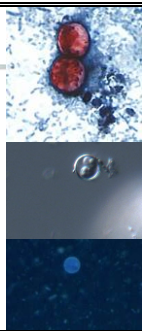
- After an average incubation period of 1 week, symptoms occur: watery diarrhea, which can be severe.
- Other symptoms include anorexia, weight loss, abdominal pain, nausea and vomiting, myalgias, low-grade fever, and fatigue.
- Untreated infections typically last for 10-12 weeks and may follow a relapsing course.



After about a week after infection, the person may manifest symptoms of watery diarrhoea which can be severe. Other symptoms include loss of appetite or anorexia, weight loss, abdominal pain, nausea and vomiting, myalgias, low grade fever and fatigue. Untreated infections usually last for about 10-12 weeks and may follow a relapsing course. Infections, especially in disease-endemic settings can be asymptomatic.

Slide  
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**Diagnosis and Treatment**



- Identification of oocysts in stool specimens by light microscopy.
- Characteristic autofluorescence
- Trimethoprim-sulfamethoxazole
- Supportive measures include management of fluid and electrolyte balance, and rest.



Diagnosis is by microscopic examination of stool and identification of the oocysts. They characteristically autofluoresce under a fluorescent microscope. The treatment is with Trimethoprim-sulfamethoxazole. Supportive treatment includes fluid and electrolyte balance and rest.

Slide

20

**Prevention/Control**



- Wash fruits and vegetables
- Be cautious when traveling to developing countries.
- Wash hands before eating and preparing food.
- Oocysts not affected by normal chlorine concentrations
- Monitoring of water used for irrigation

Why we don't look for it in U.S.

- No outbreaks associated with treated drinking water
- No good method for recovery and detection in drinking water



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The preventive measures include washing of fruits and vegetables thoroughly before consumption. Be cautious when traveling in developing countries. Always wash hands with soap and water before eating and while preparing food. Oocysts are not affected by chlorination so boil water until rolling boil to ensure killing of the cyclospora.

Slide

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**Outbreaks**



- Linked to importation of fresh produce
  - Raspberries, blackberries, fresh basil, fresh baby lettuce leaves, snow peas
- Two major outbreaks linked to raspberries from Guatemala
- Before 1996, only three outbreaks of *Cyclospora* infection had been reported in the United States.
- Between May 1 and mid-July 1996 almost 1,000 laboratory-confirmed cases were reported to the CDC.
- These infections occurred in at least 15 states and Canadian provinces and the District of Columbia.



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Cyclospora is not a big problem in the US with regards to drinking water. However, imported food and produce have been linked to cyclospora outbreaks. Particularly, raspberries, fresh basil, blackberries, fresh baby lettuce etc. Prior to 1996 only 3 outbreaks of cyclospora had been reported in the US. Between May 01 and mid July 1996 nearly 1000 lab confirmed cases of cyclospora were reported to the CDC and cyclospora came on the CDC radar.

Slide

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**Cyclospora outbreaks in 1990s**



Table 2. Documented outbreaks of cyclosporiasis in the United States and Canada in the 1990s [16, 19, 20, 22-27, 49].\*

Month and year of outbreak <sup>a</sup>	Location	No. of cases <sup>b</sup>	No. of vehicles <sup>c</sup>	Vehicle <sup>d</sup>	Source of vehicle	Comment
June or July 1988	Illinois	1	21	Water or food of food, unidentified (vehicle)	Source of vehicle	If waterborne, related to repair of water pump on 5 July; if foodborne, related to party on 29 June
May-June 1995	New York	1 <sup>e</sup>	32	Probably food		Fresh raspberries of unknown source were among the fruits served
May 1995	Florida	2	30	Raspberries <sup>f</sup>	See comment	Guatemala was a possible source
May-June 1996	US, Canada	55	145	Raspberries <sup>g</sup>	Guatemala	
March-April 1997	Florida	— <sup>h</sup>	— <sup>h</sup>	Machine	From (or US)	If evidence from December 1997 outbreak also is correct, most likely source of machine was Peru
April-May 1997	US, Canada	41	102	Raspberries <sup>h</sup>	Guatemala	
June-July 1997	Washington, D.C., and Virginia	57	341	Basil	Multiple possible sources	See text about possibility that basil was contaminated locally
Sept 1997	Florida	1	21	Fruit plate	See comment	Fruit plate might have included raspberries (see Guatemala) but not blackberries
Dec 1997	Florida	1	12	Salad (meatless)	Peru (trucks)	If evidence from March 1997 outbreak also is correct, most likely vehicle was Mexico
May 1998	Ontario	11	31	Raspberries <sup>h</sup>	Guatemala	
May 1998	Georgia	1	17	Fruit salad <sup>i</sup>	Undetermined	
May 1999	Ontario	1	104	Desert berry <sup>j</sup>	See comment	Implicated desert included fresh Guatemalan blackberries, from Chilean raspberries, and fresh US raspberries
May 1999	Florida	1	94	Probably fruit, most likely a berry <sup>k</sup>	See text	Multiple combinations of many fruits were served
July 1999	Missouri	At least 2	64	Basil <sup>l</sup>	Mexico or US	



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This table shows the documented outbreaks of cyclospora in US and Canada in the 1990's. Pay attention to the vehicle, the contaminated source, whose consumption led to outbreaks.



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### Recent Florida Outbreaks

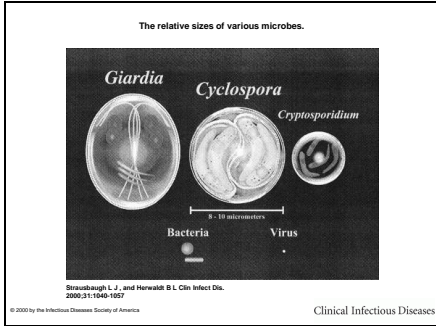
- 1996 –Palm Beach County, multiple clusters, raspberries, part of multi-state outbreak
- 1997 –Leon County –lettuce
- 1997 –Orange County –lettuce
- 1999 –Palm Beach County –undetermined (multiple fruits)
- 2005- 592 cases, fresh basil implicated in chain restaurants
- 2008- 61 cases, undetermined
- 2013-33 cases in, fresh salad mix and fresh cilantro implicated in chain restaurants



This is just to look at some outbreaks of cyclospora in FL over the last few years. As we see from the list most of the times it is associated with consumption of contaminated fresh produce.

Slide

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The relative sizes of various microbes. Three protozoan parasites are shown schematically: a Giardia lamblia cyst (length ranges from 8 to 19 μm and averages 11–12 μm), a Cyclospora cayetanensis oocyst (8–10 μm), and a Cryptosporidium parvum oocyst (average dimensions, 4.5 μm × 5 μm). The virus is not drawn to scale. The Cyclospora oocyst shown here is fully sporulated—that is, it has 2 internal sporocysts, each containing 2 sporozoites (a total of 4 infective units). Whereas oocysts of Cryptosporidium, another coccidian parasite, are fully sporulated and infectious when excreted, Cyclospora oocysts sporulate in the environment, days to weeks after excretion. Giardia, which is not a coccidian parasite, does not have sporocysts or sporozoites. (Figure courtesy of Dennis D. Juranek.)

Slide

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### Cryptosporidium spp.

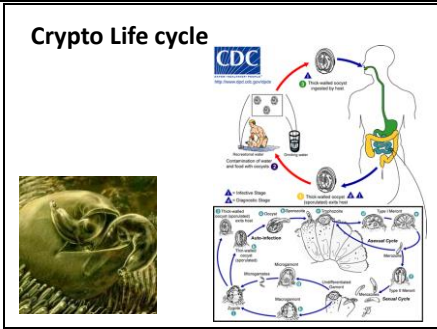
- First recognized as disease agent in 1976
- Invade and grow intracellularly in mucosal epithelial cells of the stomach and intestine
- Human infections caused by *C. hominis*, *C. parvum*
  - *C. hominis*: only human-human transmission
  - *C. parvum*: Widespread in non-human hosts: found in >155 mammalian species
- Recognized as a cause of death in AIDS patients in 1980s
- Worldwide distribution
- Does not have mitochondria or apicoplast



Cryptosporidium is another coccidian parasite that has been implicated in human diarrheal diseases. The bottom right picture shows the parasite attached to intestinal epithelium. The parasite invades and grows intracellularly in the gastric and enteric mucosa. *C. hominis* infections are exclusively transmitted amongst humans whilst *C. parvum* infection is found in more than a 150 non-human mammalian species and from them can be transmitted to humans. *C. Parvum* diarrhea is a known cause of death in AIDS patients in the 80's. Cryptosporidium is worldwide in distribution and lacks the mitochondrial and apicoplast genomes. They have only the nuclear genome.

Slide

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This is the life-cycle of cryptosporidium. Humans get infected by contaminated water ingestion as can occur at recreational facilities like Waterworld and Adventure Island. The oocyst releases sporozoites that invade the epithelial cells to form meronts in which the organism undergoes asexual reproduction to form multitudes of merozoites. These are released by rupture of the host cell and invade other epithelial cells. The meronts that give rise to merozoites by asexual reproduction are called Type I meronts. Some of the merozoites upon invasion form further Type I meronts whilst others form Type II meronts that give rise to sexual forms. The type II meront can then either give rise to a microgamete and is known as a microgamont or a macrogamete and then is known as macrogamont. The micro- and macrogamete upon release from the gamonts unite to form a zygote that gives rise to a oocyst which is passed out into the feces and is the diagnostic stage for the infection. The oocysts upon ingestion by another human are immediately infective.

Slide

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**Cryptosporidiosis**







- A wide range of manifestations, from asymptomatic infections to severe, life-threatening illness
- Incubation period averages 7 days (but can range from 2-10 days).
- **Watery diarrhea** is the most frequent symptom, and can be accompanied by dehydration, weight loss, abdominal pain, fever, nausea and vomiting.
- In immunocompetent persons, symptoms are usually short lived (1-2 weeks); they can be chronic and more severe in immunocompromised patients-severe dehydration, death
- While the small intestine is the site most commonly affected, symptomatic *Cryptosporidium* infections have also been found in other organs including other digestive tract organs, the lungs, and conjunctiva.
- Minimum infective dose – 10 to 100 oocysts depending upon species
- Estimated 3,000 cases per year in U.S. (under-estimated)

Crypto can cause a wide range of manifestations. The incubation period is about 7 days. The most common symptom is watery diarrhea. This can be accompanied by weight loss, abdominal pain, fever and nausea and vomiting.

In immunocompetent people the symptoms are usually short-lived about 1-2 weeks while they can become chronic and more severe in immunocompromised patients sometimes even leading to severe dehydration and death.

*Cryptosporidium* has sometimes been found in locations other than the intestinal tract. The minimum infective dose is very small ranging from 10-100 oocysts depending upon the species.



<p>Slide 28</p>	<p><b>Laboratory</b></p> <ul style="list-style-type: none"> <li>• Acid-fast staining methods for oocysts are most frequently used in clinical laboratories.</li> <li>• For greatest sensitivity and specificity, immunofluorescence microscopy is the method of choice (followed closely by enzyme immunoassays).</li> <li>• PCR</li> <li>• Oocysts in stool specimens (fresh or in storage media) remain infective for extended periods, and must be rendered nonviable before processing</li> </ul>  	<p>Diagnosis is done by looking for the oocysts in feces. It is rather difficult due to their small size. Use of special stains and fluorescence techniques need to be used. It is quite stable in the environment and can persist to cause infections for a long time. Therefore, it is necessary to inactivate them prior to processing them.</p>
<p>Slide 29</p>	<p><b>Treatment</b></p> <ul style="list-style-type: none"> <li>• Rapid loss of fluids because of diarrhea can be managed by fluid and electrolyte replacement.</li> <li>• Nitazoxanide has been approved for treatment of diarrhea caused by <i>Cryptosporidium</i> in immunocompetent patients.</li> <li>• For persons with AIDS, anti-retroviral therapy, which improves immune status, will also reduce oocyst excretion and decrease diarrhea associated with cryptosporidiosis.</li> </ul>  	<p>The treatment is focused on the replenishment of fluids and electrolyte balance. Nitazoxanide is the approved for treatment in immunocompetent patients. In patients with AIDS, treatment with the HAART regimen to help recover the immune status is helpful to reduce the oocyst excretion and decrease the diarrhea.</p>
<p>Slide 30</p>	<p><b>Prevention and Control</b></p> <ul style="list-style-type: none"> <li>• Thick-walled cysts can persist in environment for a long time</li> <li>• Resistant to chlorination, but filtration and ozonation ok</li> <li>• Management of watersheds where filtration not possible</li> <li>• Surveillance to keep public water supplies pathogen-free <ul style="list-style-type: none"> <li>– PCR continual monitoring</li> </ul> </li> <li>• Avoid touching animals-petting zoos, pet stores</li> <li>• Day care settings</li> </ul>  	<p>The oocysts of crypto are environmentally stable. They are resistant to chlorination but filtration and ozonation are effective. It is necessary to manage watersheds where filtration is not possible to avoid contamination of drinking water sources. Active surveillance of water sources is done by PCR to keep public water sources free of cryptosporidium. The pictures shows kids romping in a water park where they could get infected as they play in the water. Animals can host <i>C. parvum</i> so it is better to avoid petting animals in zoos and pet stores. Day care settings are also at risk of outbreaks with cryptosporidiosis.</p> <p>Recently, housing foreclosures has led to pools not being tended to. One infective bowel movement can release enough oocysts to contaminate 100 million gallons of water.</p>

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**Outbreaks**

- 1987 Carrollton, Georgia: ~13,000 people
- 1993 Milwaukee, Wisconsin: ~400,000 people
  - Spring rains and runoff from surrounding farmland had drained into Lake Michigan and overburdened the water supply system. Dairy cattle were the most likely source of this outbreak
  - Contributed to the deaths of more than 100 AIDS and chemotherapy patients.
- Non-outbreak cryptosporidiosis cases reported nationally increased from 3,411 cases in 2004 to nearly 8,300 in 2007- this mirrors the increase in the number of nationally reported cryptosporidiosis outbreaks associated with treated recreational water venues (e.g., pools, water parks, and interactive fountains)
  - Seven reported treated recreational water-associated outbreaks in 2004, 19 in 2006, and reports of 26 in 2007

These are some of the crypto outbreaks. The most important outbreak that you must know is the one that occurred in Milwaukee. The spring rains and run-off from cattle farms drained into lake Michigan and overburdened the water supply system. Dairy cattle were most likely source of this outbreak.

Epidemiologically how this outbreak was detected was by pharmacists noting a high sale of anti-diarrhea medication. This outbreak is significant as it led to death of over 100 immunocompromized individuals with AIDS and chemotherapy.



Non-outbreak cryptosporidiosis cases reported nationally surged from 2004 to 2008 in parallel with the outbreak associated cases associated with the treated recreational water venues.

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**Recent cases of Cryptosporidiosis in Florida**

Year	Reported Confirmed and Probable Cases
2001	89
2002	106
2003	128
2004	149
2005	350
2006	717
2007	738
2008	549
2009	495
2010	408

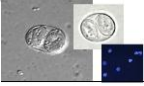
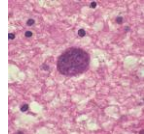


The tables gives the number of cases in FL over the previous decade as reported by the Florida Department of Health.

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**Toxoplasma gondii**





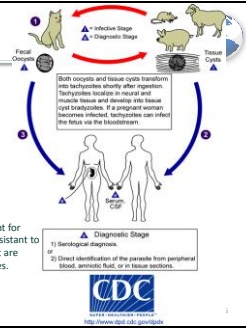

- Cats are definitive hosts, act as reservoirs
- Serologic prevalence data indicate that toxoplasmosis is one of the most common of humans infections throughout the world.
  - >500 million infected worldwide
- More than 60 million people in the U.S. carry the parasite, but very few have symptoms
  - Studies in 1988-2004 and 1999-2000 found 22.5% and 15.8% people (12-49 age) had antibodies, respectively
  - Third leading cause of death attributed to foodborne illness in the U.S.

The next parasite in this lecture is Toxoplasma gondii. This is a fairly common parasitic infection as is evident from serologic data. More than 500 million people are infected worldwide.


In the US more than 60 million people carry the parasite but very few are symptomatic. It is the third leading cause of death attributed to food-borne illness in the US.

National Health and Nutritional Assessment Survey (NHANES III)



<p>Slide 34</p>	<p><b>Transmission of Toxoplasmosis</b> </p> <ul style="list-style-type: none"> <li>• Accidental ingestion of oocysts or tissue cysts</li> <li>• Foodborne transmission <ul style="list-style-type: none"> <li>– Eating undercooked, contaminated meat (esp pork)</li> </ul> </li> <li>• Zoonotic transmission <ul style="list-style-type: none"> <li>– Accidental ingestion of oocysts</li> <li>– Exposure to cat feces</li> </ul> </li> <li>• Congenital transmission <ul style="list-style-type: none"> <li>– Woman newly infected during pregnancy</li> <li>– Severe outcomes, CNS &amp; eyes</li> </ul> </li> <li>• Transfusion and Transplants</li> </ul> 	<p>The transmission of toxoplasmosis can occur by mainly consumption of infected meat mainly pork. Zoonotic transmission by accidental ingestion of oocysts like after exposure to cat litter.</p> <p>Congenital transmission can occur from a pregnant woman to her unborn child. This type of transmission is the one with the most severe outcomes as the parasite infects the neural tissues of the brain and eyes. It can also be transmitted by transfusions and transplants.</p>
<p>Slide 35</p>	<p><b>Toxoplasma parasite stages</b> </p> <ul style="list-style-type: none"> <li>• <i>T. gondii</i> primarily exists in three forms: oocysts, tachyzoites, and bradyzoites.</li> <li>• <b>Oocysts</b> are only produced in the definitive host (cats)</li> <li>• When passed in feces and then ingested, the oocysts can infect humans and other intermediate hosts. They develop into <b>tachyzoites</b>, which are the rapidly multiplying trophozoite form</li> <li>• They divide rapidly in cells, causing tissue destruction and spreading the infection. <ul style="list-style-type: none"> <li>– Tachyzoites in pregnant women are capable of infecting the fetus.</li> </ul> </li> <li>• Eventually tachyzoites localize to muscle tissues and the CNS where they convert to tissue cysts, or <b>bradyzoites</b>.</li> <li>• Bradyzoites can cause infection if ingested in contaminated food</li> </ul> 	<p>We now talk about the <i>Toxoplasma gondii</i>'s stages. There are three stages – oocysts, tachyzoites and bradyzoites.</p> <p>Oocysts are produced in the definitive host which is the cat and passed out into its feces. When the oocysts are ingested by humans and other intermediate hosts, they develop into tachyzoites which are the rapidly multiplying trophozoite forms. They divide rapidly in cells and cause tissue destruction and spreading of the infection.</p> <p>Eventually they localize in the muscles and neural tissues of the CNS where they convert to tissue cysts called bradyzoites. Bradyzoites can cause infection if consumed in contaminated food.</p>
<p>Slide 36</p>	<p><b>Toxoplasma Life Cycle</b> </p> <ul style="list-style-type: none"> <li>• Human infection through ingestion of sporulated <i>Toxoplasma</i> oocysts or bradyzoites (tissue cysts)</li> <li>• Tachyzoites may be found in various sites throughout the body of the host.</li> <li>• Tachyzoites in macrophage cytoplasm (carried and disseminated throughout the body by infected macrophages)</li> </ul> <p>Oocysts can survive in the environment for several months and are remarkably resistant to disinfectants, freezing, and drying, but are killed by heating to 70°C for 10 minutes.</p> 	<p>This is the life cycle of <i>T. gondii</i>. Cats become infected with <i>T. gondii</i> by carnivorousism by eating rodents. After tissue cysts or oocysts are ingested by the cat, viable organisms are released and invade epithelial cells of the small intestine where they undergo an asexual followed by a sexual cycle and then form oocysts, which are excreted. The unsporulated oocyst takes 1 to 5 days after excretion to sporulate (become infective). Although cats shed oocysts for only 1 to 2 weeks, large numbers may be shed. Oocysts can survive in the environment for several months and are remarkably resistant to disinfectants, freezing, and drying, but are killed by heating to 70°C for 10 minutes. Human infection may be acquired in several ways: A) ingestion of undercooked infected meat containing <i>Toxoplasma</i> cysts ; B) ingestion of the oocyst from fecally</p>

contaminated hands or food ; C) organ transplantation or blood transfusion; D) transplacental transmission; E) accidental inoculation of tachyzoites. The parasites form tissue cysts, most commonly in skeletal muscle, myocardium, and brain; these cysts may remain throughout the life of the host.

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**Acute acquired Toxoplasmosis** 

- Generally an asymptomatic infection
- 10% to 20% of patients with acute infection may develop cervical lymphadenopathy and/or a flu-like illness.
- The clinical course is usually benign and self-limited; symptoms usually resolve within a few months to a year.
- Immunodeficient patients often have central nervous system (CNS) disease but may have retinochoroiditis, or pneumonitis.



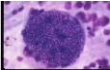





Toxoplasmosis causes a few types of clinical syndromes. The first is acute acquired toxoplasmosis. 10-20% of the infected individuals develop cervical lymphadenopathy along with a flu like illness. The clinical course is benign and self limited and the symptoms usually resolve in about a year. Immunocompromised patients have affection of the central nervous system and sometimes retinochoroiditis or pneumonitis.





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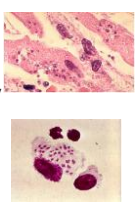


**Cerebral toxoplasmosis (AIDS)**

- In people whose immune defenses are weakened because of AIDS, cancer or immunosuppressant medication, a new toxoplasmosis infection may spread out of control and become deadly, or dormant *Toxoplasma* parasites from infection may suddenly become active again and cause severe illness.
- In patients with AIDS, toxoplasmic encephalitis is the most common cause of intracerebral mass lesions















Cerebral Toxoplasmosis occurs in people with immune compromise such as with AIDS or chemotherapy. This is due to these individuals acquiring an infections during the period of immune compromise or due to reactivation of a previously acquired infection. Patients with AIDS get mass lesions and inflammation of the brain matter called as encephalitis. The bottom image shows an arrow pointing to a mass lesion in the brain due to toxoplasma infection.

<p>Slide 39</p>	<p><b>Congenital Toxoplasmosis</b></p>  <ul style="list-style-type: none"> <li>• Results from an acute primary infection acquired by the mother during pregnancy.</li> <li>• Abortions and stillbirths after primary infection</li> <li>• 60% of infected newborns are asymptomatic (later-retinochoroiditis)</li> <li>• Hydrocephaly or microcephaly, intracranial calcifications, deafness, seizures, hepatosplenomegaly, jaundice, fever, anemia, pneumoniacerebral palsy, damage to the retina, and mental retardation.</li> <li>• Some sequelae of congenital toxoplasmosis are not apparent at birth and may become apparent years later</li> <li>• The most dangerous period to acquire toxoplasmosis is at <b>24 to 30 weeks of pregnancy</b> (10% chance of the baby being severely affected)</li> </ul> 	<p>Congenital toxoplasmosis results when a pregnant woman acquires an acute infection during the pregnancy. The pregnancy may be aborted or there may be still birth after the primary infections. About 60% of the newborns are asymptomatic at birth but later may get retinochoroiditis.</p> <p>Abnormalities of the skull and central nervous system may lead to microcephaly, hydrocephaly, intracranial calcifications. Deafness, seizures, enlargement of the liver and spleen along with fever, anemia and jaundice can result. Sometimes, pneumonia, cerebral palsy mental retardation and retinal damage may occur.</p> <p>Many a time, the sequelae manifest after a prolonged period of time sometimes years.</p> <p>The most dangerous time for a pregnant woman to acquire the infection is 24-30 weeks, as there is a 10% chance of the baby being severely affected.</p>
<p>Slide 40</p>	<p><b>Retinochoroiditis</b></p>  <ul style="list-style-type: none"> <li>• Inflammation of the retina and choroid (thick vascular area at back of eye), can result from congenital infections or from acute or reactivated infections acquired postnatally.</li> <li>• With congenital infection, retinochoroiditis can develop weeks to years after birth.</li> <li>• The lesions are focal in nature and generally self-limiting.</li> <li>• Symptoms can include blurred vision or other visual defects</li> <li>• The disease is rarely progressive in immunocompetent individuals, but can scar the retina.</li> <li>• The disease can be quite severe in AIDS patients and continue to progress.</li> </ul> 	<p>This is one of the significant manifestations of toxoplasma infection. There is inflammation of the retina and the vascular coat of the eye-choroid. This can result from congenital infections or from acute infections or reactivated infections acquired after birth. In congenital infection, the retino-choroiditis may manifest years later. The lesions are focal and are generally at the center of the macula. Macula is the point of greatest concentration of the cone cells in the retina and thus responsible for sharp vision. Not surprisingly, inflammation there leads to blurred vision. Healing occurs with scarring and permanent decrease in visual acuity. The infection reactivates when the immunity is compromised.</p> <p>In AIDS patients the disease can be progressive and severe.</p>

<p>Slide 41</p>	<div data-bbox="219 100 657 430"> <h3>Laboratory Diagnosis</h3> <ul style="list-style-type: none"> <li>• Serologic testing is the routine method of diagnosis</li> <li>• Observation of parasites in patient specimens, such as bronchoalveolar lavage material from immunocompromised patients, or lymph node biopsy.</li> <li>• Isolation of parasites from blood or other body fluids, by intraperitoneal inoculation into mice or tissue culture.</li> <li>• Detection of parasite genetic material by PCR, especially in detecting congenital infections in utero.</li> </ul>   </div>	<p>Lab diagnosis of toxoplasmosis is more challenging. It is not easy to detect the oocysts. The common method of diagnosis by serology for the IgM and IgG antibodies. IgM antibodies are detected in an acute infections whilst IgG antibodies are indicative of an old infection. In patients with pneumonitis, bronchoalveolar lavage can detect the parasites as can biopsies of lymph nodes as seen in the top image.</p> <p>Sometimes isolation of parasites from blood and other body fluids by intra-peritoneal inoculation in mice or tissue culture is done.</p> <p>For diagnosis of infection in utero PCR is done to detect parasite genetic material.</p>
<p>Slide 42</p>	<div data-bbox="219 821 657 1150"> <h3>Prevention of Toxoplasmosis</h3> <p><b>Environmental Exposures</b></p> <ul style="list-style-type: none"> <li>• Avoid drinking untreated drinking water, particularly when traveling in less developed countries.</li> <li>• Avoid contact with soil or sand that is contaminated with cat feces</li> <li>• Feed cats only canned or dried commercial food or well-cooked table food, not raw or undercooked meats.</li> <li>• Cats infested with <i>Toxoplasma</i> typically shed infective eggs for one period of 3 - 21 days during their lives.</li> <li>• If you are pregnant or immunocompromised: <ul style="list-style-type: none"> <li>– Avoid changing cat litter if possible.</li> <li>– Keep cats indoors.</li> <li>– No new kitties</li> </ul> </li> </ul>  </div>	<p>Prevention measures for toxoplasmosis include avoidance of consumption of untreated water especially in developing travel. Avoid contact with soil or sand contaminated with cat feces.</p> <p>Cats should be fed only commercially available cat feed or fully cooked meat if feeding them table scraps. Cats infected with toxoplasmosis shed the oocysts for one period of 3-21 days in their lifetime.</p> <p>Pregnant women should avoid changing cat litter if possible and keep the cats indoors and avoid getting new pet cats.</p> <p>Keep outdoor sandboxes covered.</p> <p>In the United States, people are much more likely to become infected through eating raw meat and unwashed fruits and vegetables than from handling cat feces.</p>



<p>Slide 43</p>	<p><b>Prevention-Foodborne</b> </p> <ul style="list-style-type: none"> <li>• Cook food to safe temperatures. <ul style="list-style-type: none"> <li>– Beef, lamb, and veal roasts and steaks should be cooked to at least 145°F throughout.</li> <li>– Pork, ground meat, and wild game should be cooked to 160°F.</li> <li>– Whole poultry should be cooked to 180°F in the thigh.</li> </ul> </li> <li>• Peel or wash fruits and vegetables thoroughly before eating.</li> <li>• Freeze meat for several days before cooking to greatly reduce chance of infection.</li> </ul> 	<p>Further given the risk of toxoplasmosis from uncooked meats and contamination of raw foods, all meats should be thoroughly cooked to kill tissue parasites.</p> <p>Fruits and vegetables should be washed thoroughly and peeled before eating.</p> <p>Meat may be frozen for several days before cooking to reduce chance of infection.</p>
<p>Slide 44</p>	<p><b>Tx</b> </p> <ul style="list-style-type: none"> <li>• Treatment is not needed for a healthy person who is not pregnant.</li> <li>• Treatment may be recommended for pregnant women or persons who have weakened immune systems.</li> <li>• Sulfadiazine + pyrimethamine or clindamycin + pyrimethamine</li> </ul> 	<p>Non pregnant healthy individuals do not need treatment. Treatment is indicated for symptomatic individuals and is with a combination of sulfadiazine and pyrimethamine or clindamycin and pyrimethamine.</p> <p>Symptoms will usually go away within a few weeks.</p>
<p>Slide 45</p>	<p><b>Toxoplasma and Human Behavior</b> </p> <ul style="list-style-type: none"> <li>• Chronic infection causes subtle behavior changes in people? <ul style="list-style-type: none"> <li>– Reaction time is affected</li> <li>– Women seem to become more intelligent, outgoing, conscientious, sexually promiscuous, and kind; changes in men seem to cause opposite trends.</li> </ul> </li> <li>• <i>Toxoplasma gondii</i> tend to encyst in the amygdala of the brain (controls emotions-responses to social situations, sexual responses, aggression, fear, anxiety)</li> <li>• Infected mice have increased activity levels and show increased aggression, and rats show a marked decrease in their natural fear of cat odors (more likely to be eaten by a cat?)</li> </ul> 	<p>This is something that I think is pretty interesting.</p> <p>Some believe that the infection with this parasite increases the reaction time. Women seem to become more intelligent, outgoing, conscientious, sexually promiscuous and kind and in men seems to cause opposite trends.</p> <p>This may be because, toxoplasma tends to encyst in parts of the brain- amygdala- that controls emotions and responses to social situations, sexual responses, aggression, fear and anxiety.</p> <p>In experiments, mice have increased activity and show aggression. This is accompanied by a marked lack of fear of cat odors. Maybe the parasite makes the rat be eaten by the cat and thus help reach its definitive host.</p>

<p>Slide 46</p>	<div data-bbox="217 100 651 422"> <p><b>Cause for Concern?</b></p> <ul style="list-style-type: none"> <li>• Mental disorders <ul style="list-style-type: none"> <li>– Studies show that people with schizophrenia and other mental health problems have a higher incidence of exposure to <i>T. gondii</i> than healthy controls</li> <li>– Antipsychotic drugs commonly used for people show activity against <i>T. gondii</i> parasites and infected rodents show reduced behavioral changes when treated with these drugs.</li> <li>– Since 1953, a total of 19 studies of <i>T. gondii</i> antibodies in persons with schizophrenia and other severe psychiatric disorders and in controls have been reported</li> <li>– UK (2009)-toxoplasmosis parasite is statistically linked to the development of schizophrenia and other psychoses - by producing the enzyme tyrosine hydroxylase, which increases the production of L-DOPA, the precursor of dopamine in the brain.</li> </ul> </li> <li>• Organ, bone marrow transplantations</li> <li>• Blood transfusion</li> </ul>  </div>	<p>Studies have shown that Schizophrenia and other psychiatric conditions have a greater prevalence in individuals with toxoplasma infections. Also, serological studies shows higher prevalence of anti-toxoplasma antibodies in individuals with psychiatric disorders.</p> <p>In 2009 toxoplasmosis was statistically linked to development of schizophrenia and other psychoses by producing the enzyme tyrosine hydroxylase which increases the production of L-DOPA, the precursor of dopamine in the brain.</p> <p>Another concern is the possible transmission in organ and bone marrow transplants and through blood transfusion.</p>
<p>Slide 47</p>	<div data-bbox="217 852 651 1173"> <p><b>Outbreaks</b></p> <ul style="list-style-type: none"> <li>• Outbreaks associated with drinking water <ul style="list-style-type: none"> <li>– First documented outbreak associated with municipal water supply: British Columbia (1995)-100 cases</li> <li>– Brazil (2001-2002)-155 cases</li> <li>– India (2001-2004)-178 ocular toxoplasma cases</li> </ul> </li> <li>• Unknown source <ul style="list-style-type: none"> <li>– Turkey, 2002: 171 cases</li> </ul> </li> </ul>  </div>	<p>Toxoplasmosis can be asymptomatic. Outbreaks are rare and can occur with contamination of drinking water. These are some of the outbreaks that have occurred in the past.</p>