Intestinal Ciliates, Coccidia (Sporozoa) and Microsporidia

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<th>Balantidium coli (Ciliates)</th>
<th>Cyclospora cayetanensis (Coccidia, Sporozoa)</th>
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<td>Enterocytozoon bieneusi (Microsporidia)</td>
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<td>Isospora belli (Coccidia, Sporozoa)</td>
<td>Encephalitozoon intestinalis (Microsporidia)</td>
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**Balantidium coli**

**Introduction:**

*Balantidium coli* is the only member of the ciliate (Ciliophora) family to cause human disease (*Balantadiasis*). It is the largest known protozoan causing human infection.

**Morphology:**

**Trophozoites:** Trophozoites are oval in shape and measure approximately 30-200 μm. The main morphological characteristics are cilia, 2 types of nuclei; 1 macro (Kidney shaped) and 1 micronucleus and a large contractile vacuole. A funnel shaped cytosome can be seen near the anterior end.

**Cysts:** The cyst is spherical or round ellipsoid and measures from 30-70 μm. It contains 1 macro and 1 micronucleus. The cilia are present in young cysts and may be seen slowly rotating, but after prolonged encystment, the cilia disappear.

**Life Cycle:**

Definitive hosts are humans including a main reservoir are the pigs (It is estimated that 63-90 % harbor *B. coli*), rodents and other animals with no intermediate hosts or vectors. Cysts ( Infective stage) are responsible for transmission of balantidiasis through ingestion of contaminated food or water through the oral-fecal route. Following ingestion, excystation occurs in the small intestine, and the trophozoites colonize the large intestine. The trophozoites reside in the lumen of the large intestine of humans and animals, where they replicate by binary fission. Some trophozoites invade the wall of the colon and
multiply. Trophozoites undergo **encystation** to produce infective cysts. Mature cysts are passed with feces.

**Epidemiology:**

Worldwide distribution.

Because **pigs are the main animal reservoir**, human infections occur more frequently in areas where pigs are raised.

Other potential animal reservoirs include **cows, horses rodents and nonhuman primates**. The infection occurs mostly in farm workers by ingestion of cysts in fecal material of farm animals (**Zoonotic protozoan**).

Man-to-man transmission is rare but possible.

**Pathogenity:**

Most cases are asymptomatic. If symptomatic, *B. coli* infections may cause severe infection that resemble **amoebiasis**. Symptoms include diarrhea, nausea, vomiting and anorexia (loss of appetite) and even **dysentery**. The diarrhea may persist for long periods of time resulting in acute fluid loss and weight loss.

*Balantidium coli* also has the potential to **penetrate the mucosa resulting in ulceration** just as those of *Entamoeba histolytica*. **Metastatic and extraintestinal** diseases, liver, lung and brain abscesses, usually are very rare.

**Diagnosis:**

1. **Fresh stool samples**: In saline wet mounts, trophozoites can be easily recognized because of **their size and rapid revolving rotation**, cysts are seen less frequently. In a stained preparation, the characteristic **macro and micronuclei** can be observed.
2. **Concentrates** then follow as above in wet mount.
3. *B. coli* can also be seen in intestinal tissue stained with hematoxylin and eosin.

### Treatment:

<table>
<thead>
<tr>
<th>Infection</th>
<th>Drug</th>
<th>Adult dosage</th>
<th>Pediatric dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balantidiasis (Balantidium coli)</td>
<td>Tetracycline¹⁴</td>
<td>500 mg bid x 10d</td>
<td>40 mg/kg/d (max. 2 g) in 4 doses x 10d</td>
</tr>
<tr>
<td>Alternatives:</td>
<td>Metronidazole⁷</td>
<td>750 mg tid x 5d</td>
<td>35-50 mg/kg/d in 3 doses x 5d</td>
</tr>
<tr>
<td></td>
<td>Iodoquinol⁷</td>
<td>650 mg tid x 20d</td>
<td>40 mg/kg/d in 3 doses x 20d</td>
</tr>
</tbody>
</table>

Note: Tetracyclines usage is **contraindicated** in pregnancy and in children <8 years old.

**Coccidia**

The coccidia class are characterized by a thick walled **oocyst** stage that is typically excreted with feces. These organisms reside within the phylum *Apicomplexa*, along with other protozoan parasites such as *Plasmodium* species that characterized by **complex life cycles**. The most important gastrointestinal coccidian are *Cryptosporidium*, *Cyclospora* and *Isospora* carry out their entire life cycle within the **intestinal epithelial cells** of the host and are **transmitted** by the fecal-oral route.

*Cryptosporidium parvum* and *C. hominis*

*Cryptosporidium* contribution to gastrointestinal disease was first described in 1976 but without any concern. It was until the 1980s, the scores of cases were described increasingly among patients with acquired **immunodeficiency syndrome (AIDS)**. The disease gained greater notification after a **massive outbreak of water-borne cryptosporidial infection in Milwaukee, Wisconsin in 1993** that affected more than 400,000 people.

Nowadays, *Cryptosporidium* species were recognized **worldwide**, occurring in both developed and developing countries and causing infection in both humans and their livestock. *Cryptosporidium parvum* is the species responsible for human infection including a **wide range of hosts**. However, *C. hominis* is almost exclusively a parasite of humans, which is differentiated from *C. parvum* through **genetic analysis**.

**Life Cycle:**

Definitive hosts are humans including a wide range of animals with **no intermediate hosts or vectors**. Infection occurs by the ingestion of sporulated oocysts, containing **4 sporozoites ( Infective stage)** from contaminated water, food, or by the fecal-oral route and possibly inhalation. **Excystation** releases sporozoites that parasitize epithelial cells of the gastrointestinal tract or the respiratory tract. In epithelial cells, the
parasites undergo asexual schizogony (Multiple nuclear division followed by the cytoplasmic division) and/or merogony (Partial development) multiplication and then sexual multiplication (gametogony) producing microgamonts (male) and macrogamonts (female). Upon fertilization of the microgamonts by the microgametes, oocysts developed that sporulate in the infected host. Two different types of oocysts are produced, a) the thick-walled, sporulated oocyst, containing 4 sporozoites, which is commonly excreted through feces and possibly respiratory secretions, and b) the thin-walled oocyst, which is primarily involved in autoinfection. Oocysts are infective upon excretion, thus direct and immediate fecal-oral transmission is possible.

**Epidemiology:**

The reported prevalence of infection varies widely and is influenced by geographic region, age, immune status, local outbreaks and the different diagnostic methods.
Transmission for *Cryptosporidium* are similar to other fecal-oral diseases, water and occasionally food sources. However, waterborne cryptosporidiosis outbreaks have been especially notable.

Factors that contribute to the increased risks of *Cryptosporidium* waterborne outbreaks are:

1. **Small size of oocysts.**
2. **Monoxenous** (Single Host Life Cycle) development.
3. Close associations between human and animal hosts (**Zoonosis**).
4. **Large number of oocysts excreted** (Up to 100 billion per calf).
5. **Low infective dose.**
6. **Robust oocysts** which are resistant to chlorine.
7. **Infectious sporulated oocysts** excreted.

Despite the notable waterborne outbreaks, **Zoonotic** (*C. parvum*) and human-to-human (*C. hominis*) transmission appears to **predominate** especially in **travelers** and amongst **daycare personnel** working with young children.

Health care workers should be aware of the potential for **nosocomial** (hospital-acquired) transmission.

**Pathogenity:**

This organism can be found throughout the gastrointestinal tract; however it appears to have an **affinity for epithelial cells in the jejunum, ileum and proximal colon.**

Infection with *Cryptosporidium* sp. range from **asymptomatic** infections to severe, **life-threatening illness**; incubation period is an average of 7 days.

**Watery diarrhea** is the **most frequent symptom**, and can be accompanied by dehydration, steatorrhea, headache, weight loss, abdominal pain, fever, nausea and vomiting.

In immunocompetent persons, **diarrheal symptoms** are usually short up to 2 weeks.

In immunocompromised (AIDS) patients, chronic diarrhea is often **watery and lasting for months or even years** and can lead to dehydration requiring hospitalization. **AIDS patients with CD4 counts <200/µl** reveals more severe and chronic symptoms; while the small intestine is the site most commonly affected, symptomatic *Cryptosporidium* infections have also been found in other organs including biliary tract, pancreas, liver, lungs, and possibly **conjunctiva.**

**Diagnosis:**

1) Routine stool O&P examination will **generally fail** to detect the organism and the clinician should specify that *Cryptosporidium species* for the differential diagnosis.
The sample should be **preserved in 10% buffered formalin** (Or concentration method with **formalin-ethyl acetate fixation**) to reduce the risk to laboratory workers.

Wet mount examination with iodine can be used with specimens containing moderate to high numbers of oocysts.

To **maximize recovery of oocysts**, stool samples should be **concentrated** prior to microscopic examination. **Formalin-ethyl acetate sedimentation** is the recommended **stool concentration method**. **Sucrose flotation concentration** method is an alternative method that can be used for clinical laboratories.

Standard staining include **modified acid fast staining** in which oocysts appear pink or red round oocysts of 4-5 µm in diameter on a blue or blue-green background.

**Sporozoites (Each oocyst may have 4 sporozoites) may be seen in individual oocysts** and their visualization may assist in the diagnosis.

2) Immunodiagnosis (ELISA).
3) Direct fluorescent antibody (DFA) assay (See next figure).
4) Molecular analysis by PCR-based assays.

<table>
<thead>
<tr>
<th>Oocysts of C. parvum, in wet mount</th>
<th>Oocysts of C. parvum direct fluorescent antibody</th>
<th>C. parvum stained by the modified acid-fast method</th>
<th>C. parvum with fluorescent stain auramine rhodamine</th>
</tr>
</thead>
</table>

**Treatment:**

No approved **effective treatment** for *Cryptosporidiasis* infection though paromomycin is used as a drug of choice with **Non-HIV patients**.

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<th>Drug</th>
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<th>Pediatric dosage</th>
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<tbody>
<tr>
<td>Cryptosporidiosis (Cryptosporidium)</td>
<td>Nitazoxanide</td>
<td>500 mg bid x 3d</td>
<td>1-3yrs: 100 mg bid x 3d</td>
</tr>
<tr>
<td>HIV infected</td>
<td>None is approved</td>
<td>4-11yrs: 200 mg bid x 3d</td>
<td></td>
</tr>
</tbody>
</table>
Isospora (Syn. Cystoisospora) belli

Isospora belli is believed to be a valid species which ONLY infects humans causing coccidiosis. However, it is the least common of the three intestinal coccidia that infect humans. It has a worldwide distribution but is more common in tropical regions and areas with poor sanitation. Infections are often asymptomatic, and those with symptoms tend to be self-limiting with a duration of a few weeks. Infections are more common and the symptoms more severe in AIDS patients.

Life cycle:
Similar to Cryptosporidium sp. in having a complex life cycle with sexual and asexual stages. It could be the main difference is the excretion of immature oocyst with one sporoblast (Rarely two). After excretion maturation, 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. Therefore, each oocyst have 8 sporozoites.

Pathogenity:
In general, the symptoms are similar to those of cryptosporidiosis. Symptoms associated with I. belli infection include diarrhea, steatorrhea, headache, fever, crampy abdominal pain, nausea, dehydration and weight loss.

Another common finding among AIDS patients is a chronic intermittent diarrhea lasting for months to years. There have also been a few reports of disseminated extra-intestinal isosporiasis in AIDS patients.

Eosinophilia may be present (differently from other protozoan infections).

Diagnosis:
Isosporiasis is diagnosed by the identification of the typical ellipsoidal shape oocysts (large 25 to 30 µm) in the feces (Stool O&P) or duodenal or jejunal aspirate using:

1- For safety measures, samples can be fixed in 10% formalin before direct microscopy, concentration procedures, and preparation of stained smears.

2- Wet mount samples can be observed with a) bright-field, b) differential interference contrast (DIC), and c) epifluorescence microscopy. They can also be stained by modified acid-fast stain or other stains.
3- **Storage medium** composed of aqueous potassium dichromate (2.5% w/v, final concentration) that could be used to monitor sporation & maturation of oocysts.

4- **Concentration methods** are recommended as the oocysts may be passed in small amounts and intermittently.

5- Molecular analysis by PCR-based assays for research.

**Treatment:**

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</thead>
<tbody>
<tr>
<td><em>Isospora belli</em></td>
<td>Trimethoprim-sulfamethoxazole</td>
<td>TMP 160 mg/SMX 800 mg (1 DS tab)</td>
<td>TMP 5 mg/kg, SMX 25 mg/kg bid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bid x 10d</td>
<td>x 10d</td>
</tr>
</tbody>
</table>

**Cyclospora cayetanensis**

In 1994, this organism was confirmed to be a coccidian parasite. Since that time it has been associated with many foodborne outbreaks and traveller’s diarrhea. Cyclosporiasis has been reported in many countries, but is most common in tropical and subtropical areas.

**Life cycle, pathogenity and treatment** is similar to *Isospora belli* and *Cryptosporidium* Sp. except that the unsporulated *Cyclospora cayetanensis* at the time of excretion are NOT infective until sporulation is completed. In addition, the oocyst contains two sporocysts which each contain two sporozoites.

**Diagnosis:**

Is similar to *Isospora belli* diagnostics with more beneficial Sporulation Assay.
Sporulation Assay:

Because of the morphologic similarity between freshly passed, unsporulated *Cyclospora* oocysts and blue-green algae (cyanobacterium-like bodies), it has been advocated that to confirm the diagnosis of cyclosporiasis.

This is accomplished by placing an aliquot of fresh stool in 2.5% potassium dichromate (which reduces bacterial overgrowth), and keeping it under observation for over 2- to 3-week period for evidence of sporulation of the oocysts.

![Sporulation Assay](image)

Microscopy:

In wet mounts, *Cyclospora* oocysts appear as round organisms, 8 to 10 µm in diameter, with a distinct oocyst wall.

![Microscopy](image)

<table>
<thead>
<tr>
<th>Species</th>
<th>Excreted Form</th>
<th>Size (µm)</th>
<th>Oocyst Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cryptosporidium</em></td>
<td>Sporulated oocysts</td>
<td>4-5</td>
<td>4 sporozoites, no sporocysts</td>
</tr>
<tr>
<td><em>Cyclospora</em></td>
<td>Unsporulated oocysts</td>
<td>8-10</td>
<td>2 sporocysts with 2 sporozoites each</td>
</tr>
<tr>
<td><em>Isospora</em></td>
<td>Unsporulated oocysts</td>
<td>30 x 12</td>
<td>2 sporocysts with 4 sporozoites each</td>
</tr>
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In summary:

Coccidians in Human feces have the following differential characteristics: