Medical Laboratory Techniques Department
Lecture 6 : Medical parasitology
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Coccidia

The coccidia are unicellular protozoa and belong to the **Phylum**: Apicomplexa.

- They live intracellularly, at least during a part of their life cycle and at some stage in their life cycle, they possess a structure called the **apical complex**, by means of which they attach to and penetrate host cells; hence included in Phylum Apicomplexa.
- All coccidian have a **sexual sporogonic phase** and an **asexual schizogonic phase**.
- Many of them also show an alteration of hosts; a definitive host and an intermediate host.
- Many parasites considered in this chapter have acquired great prominence due to their frequent association with HIV infection.

Intestinal coccidian species

1. *Cryptosporidium parvum* مهم

Two species of Cryptosporidium, C. hominis and C. parvum mostly cause human infections.

Habitat

C. parvum inhabits the small intestine. It may also be found in stomach, appendix,colon, rectum, and pulomonary tree.

Morphology

The infective form of the parasite is oocyst.

- The oocyst is spherical or oval and measures about 5 μm in diameter.
- Oocysts do not stain with iodine and is acid fast.
- The wall of the oocysts is thick, but in 20% cases, wall may be thin. These thin walled oocysts are responsible for autoinfection.
- Both thin walled and thick walled oocyst contain 4 crescent shaped **sporozoites.**
- Oocyst can remain viable in the environment for long periods, as it is very hardand resistant to most disinfectants and temperature up to 60°C.
- It can survive chlorinated water, but sequential application of ozone andchlorine has been found effective in eliminating the cysts.

Life Cycle

The parasite complete its life cycle, sexual and asexual phases in a single host (monoxenous)

Suitable host: Man.

Reservoirs: Man, cattle, cat, and dog.

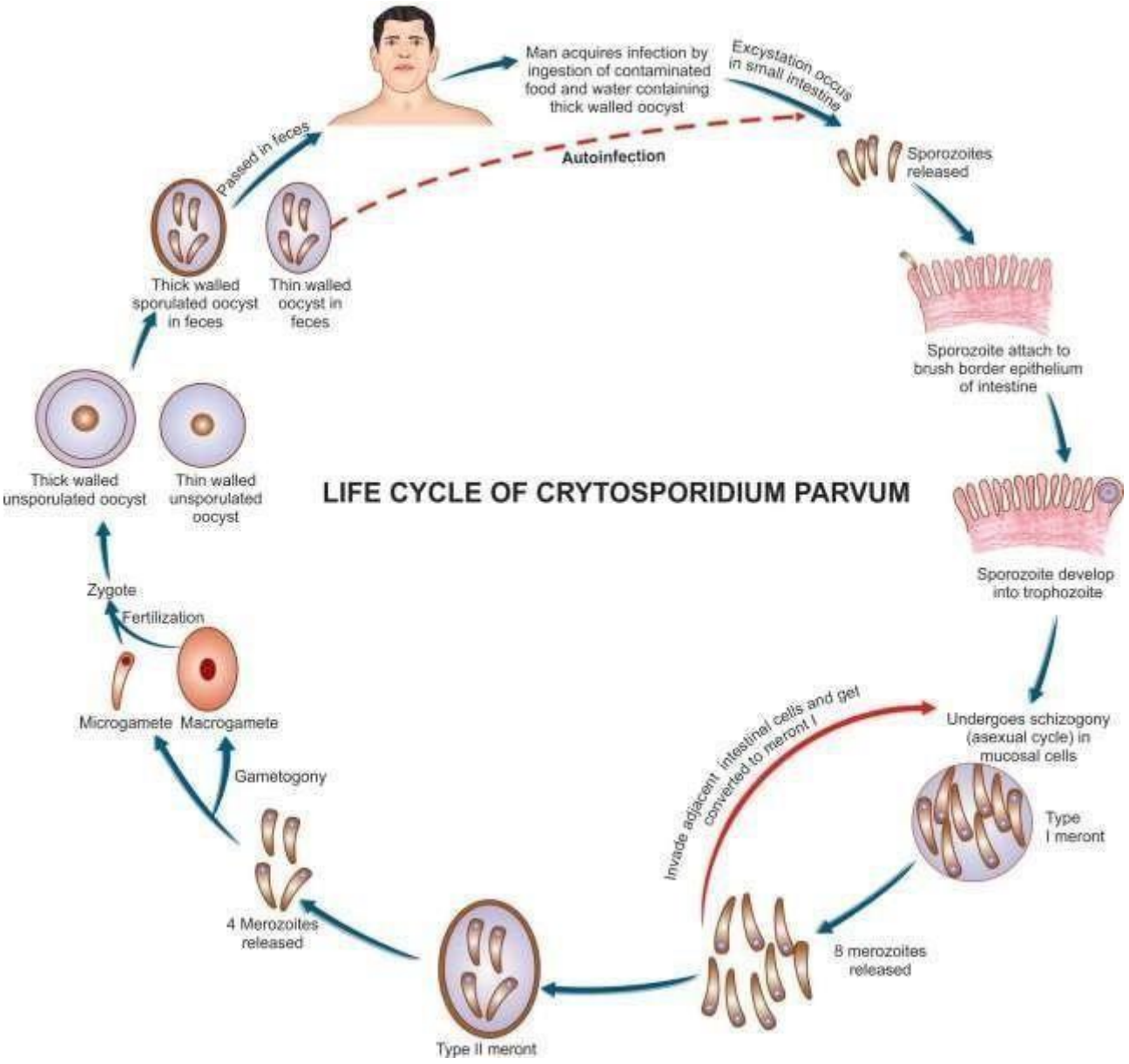
Mode of transmission

Man acquires infection by:
Ingestion of food and water contaminated with feces containing oocysts
Autoinfection.
Infective form: Sporulated oocysts.

- The oocyst contains 4 sporozoites, which are released in the intestine.
- The sporozoites develop into trophozoites within parasito phorous vacuoles in the brush border of the intestine.
- The trophozoites undergo **asexual** multiplication (**schizogony**) to produce **type I meronts**.
- Eight merozoites are released from each type I meront. These merozoites enter adjacent epithelial cells to repeat schizogony or form type II meronts, which undergo gametogony.
- Four merozoites are released from each type II meront. The merozoites enter host cell to form sexual stages: **microgamete** and **macrogamete**.
- After fertilization, the zygote formed develops into the oocyst. The oocysts undergoes sporogony to form spourlated oocyst, which contain 4 sporozoites. Spourlated oocysts are released into the feces and transmit the infection from one person to another. Some of the oocysts have a thin wall surrounding 4 sporozoites and are called as **thin-walled oocysts**. These oocysts infect the same host and maintain the cycle of **autoinfection**.
- The oocysts are fully mature on release and are infective immediately without further development.

Pathogenicity and Clinical Features

- Humans get infection either by ingestion of contaminated food and water with feces or by direct contact the infected animals. Human to human transmission can also occur. Incubation period is 2–14 days.
- Clinical manifestations of *C. parvum* infection vary depending upon the immune status of the host. Infection in healthy immunocompetent persons may be asymptomatic or cause a self-limiting febrile illness, with watery diarrhea in conjunction with abdominal pain, nausea, and weight loss. It can also cause childhood and traveller's diarrhea, as well as waterborne outbreaks. In immunocompromised hosts, especially those with AIDS and CD4+ T cell counts below 100/uL, diarrhea can be chronic, persistent, and remarkably profuse, causing signify cant fluid and electrolyte depletion, weight loss, emaciation, and abdominal pain. Stool volume may range from 1 to 25 L/day. Billary tract involvement can manifest as right upper quadrant pain, sclerosing cholangitis, or cholecystitis.



Life cycle of *C. parvum*

Laboratory Diagnosis

a. Stool Examination

Diagnosis is made by demonstration of the oocysts in feces.

A direct wet mount reveals colorless, spherical oocyst of 4–5 µm, containing large and small granules.

The staining can also be used for demonstration of oocysts in other specimens like sputum, bronchial washing, etc.

b. Histopathological Examination

Cryptosporidia can also be identified by light and electron microscopy at the apical surface of intestinal epithelium from biopsy specimen of the small bowel (jejunum being the preferred site).

c. Serodiagnosis

Antibody specific to *C. parvum* can be demonstrated within 2 months of acute infection.

- Antibody persists for at least an year and can be demonstrated by ELISA or immunofl uroscence.
- An ELISA for detection of *cryptosporidium* antigens in stools using monoclonal antibody has also been developed and is highly sensitive and specific.

d. Molecular Diagnosis

For seroepidemiological study, western blot technique is employed by using a 17KDA and 27KDA sporozoite antigen.

- PCR technique has also been applied to detect viable cysts.

2. Isospora belli

Morphology

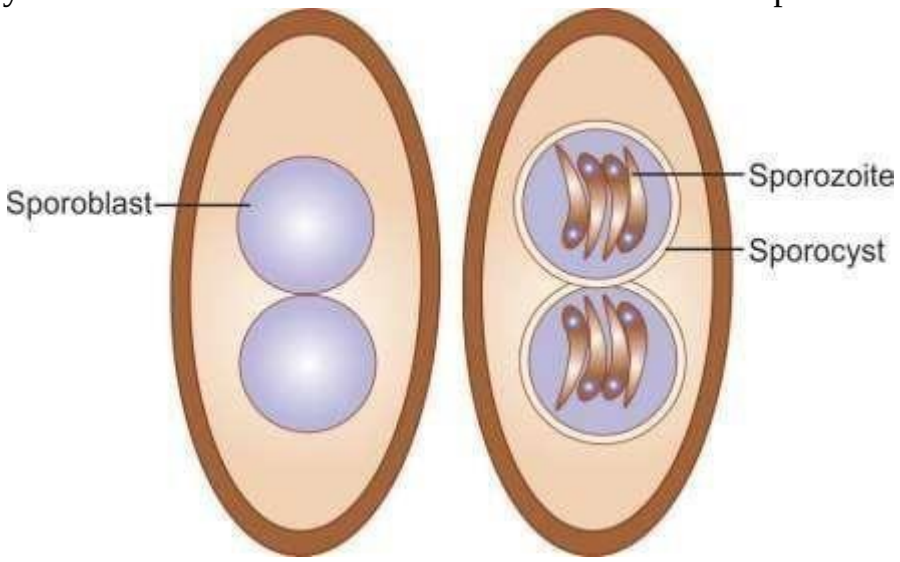
Oocysts of *I. belli* are elongated ovoid and measure 25 µm × 15 µm.

- Each oocyst is surrounded by a thin smooth 2 layered cyst wall.
- Immature oocyst seen in the feces of patients contains two sporoblasts.
- The oocysts mature outside the body.
- On maturation, the sporoblast convert into **sporocysts**. Each sporocyst contain 4 crescent shaped sporozoites.
- The **sporulated oocyst containing 8 sporozoites** is the **infective stage** of the parasite.

Life Cycle

I. belli completes its life cycle in one host.

- Man gets infection by ingestion of food and water contaminated with sporulated oocyst.
- When a sporulated oocyst is swallowed, 8 sporozoites are released from the 2 sporocysts in the small intestine and invade the intestinal epithelial cells.



A **B**

Oocysts of *Isospora belli.* A. Immature cyst; B. Mature cyst

- In the epithelium, the sporozoites transform into trophozoites, which multiply asexually (**schizogony**) to produce a number of (**merozoites**). The merozoites invade adjacent epilhelial cells to repeat asexual cycle.
- Some of the trophozoites undergo sexual cycle (***gametogony***) in the cytoplasm of enterocytes and transform into **macrogametocytes** and **microgametocytes**.
- After fertilization, a *zygote* is formed, which secretes a cyst wall and develops into an **immature oocyst**.
- These immature oocysts are excreted with feces and mature in the soil.
- **Incubation period:** 1–4 days.

Clinical Features

Infection is usually asymptomatic.

- Clinical illness includes abdominal discomfort, mild fever, diarrhea, and malabsorption.
- The diarrhea is usually watery and does not contain blood or pus and is self-limiting.
  However, protracted diarrhea, lasting for several years can be seen in immunocompromised persons, particularly in the human immunodeficiency virus (HIV) infected.

Laboratory Diagnosis

a. Stool Examination

Indirect evidence

- High fecal fat content.
- Presence of fatty acid crystals in stool.
- Presence of Charcot Leyden crystals in stool.

Direct evidence

It may be difficult to demonstrate the transparent oocyst in saline preparation of stool.

- Stool concentration techniques may be required when direct wet mount of stools are negative.
- The staining technique used are Modified ZiehlNeelsen stain or Kinyoun acid fast staining of stool smear. In these methods, pink colored acid fast large oocyst (>25 μm) can be demonstrated. The stool smear can also be stained by auramine rhodamine and Giemsa stains.

b. Duodenal Aspirates

After repeatedly negative stood examinations, duodenal aspirate examination or entero test can be performed to demonstrate oocyst.

c. Intestinal Biopsy

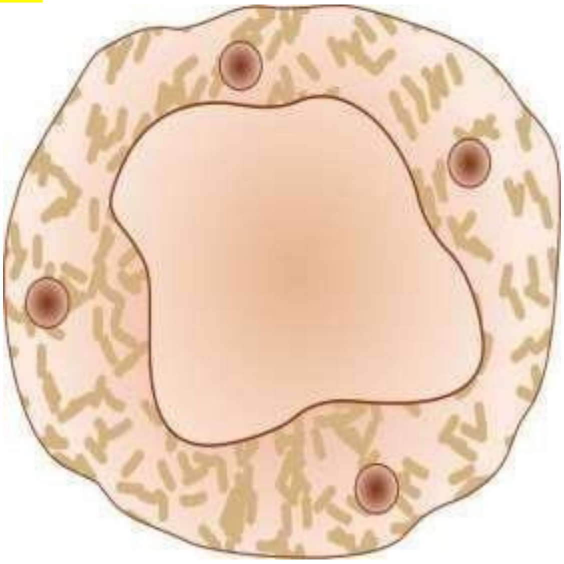
Upper gastrointestinal endoscopy may provide biopsy specimens for demonstration of oocysts.

d. Others

Eosinophilia, which is generally not seen with other enteric protozoan infections, is detectable in case of isosporoloasis.

3. *Blastocystis Hominis* مهم

Blastocystis hominis was previously سابقًا considered a yeast, but recently it has been reclassified as a protozoan.



Habitat

It is a strict اللاهوائي تماما anaerobic protozoa found in large intestine of humans.

Morphology

B. hominis has 3 morphological forms:

- **Vacuolated form** is usually seen in stool specimen. It measures 8 µm in diameter and is characterized by its large central vacuole, which pushes the cytoplasm and the nucleus to the periphery. It multiplies by binary fission.
- **Amoeboid form** is a polymorphrous cell slightly larger than the vacuolated form occasionally seen in the feces. It multiplies by sporulation.
- **Granular form** measures 10–60 µm in diameter and is seen exclusively in old cultures.

Pathogenicity and Clinical Features

The pathogenecity of *B. hominis* is doubtful مبهمة. However, recent studies have shown the parasite to be associated with diarrhea.

Clinical manifestations include diarrhea, abdominal pain, nausea, vomiting and fever .

More than half of the patients suffering from infection with *B. hominis* have been found to be immunologically compromised.

Diagnosis

The condition is diagnosed by demonstration of the organism in stool smear stained by Giemsa or iron hematoxylin or Trichrome stains.

4. Sarcocystis

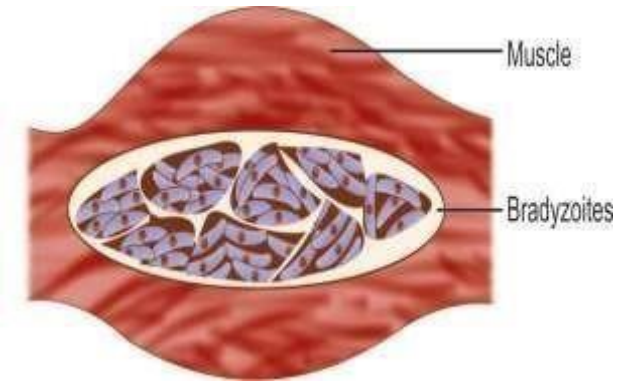
Three species of genus *Sarcocystis* can infect humans

- *S. hominis* (transmitted through cattle).
- *S. suihominis* (transmitted through pig).
- *S. lindenmani*.
- Humans are the definitive host of *S. hominis* and *S. suihominis* and the intermediate host for *S. lindenmani*.
- *Sarcocystis* species produce cyst in the muscle of the intermediate hosts. These cysts, called *Sarcocysts* contain numerous merozoites (**bradyzoites**).
- When sarcocyst is eaten by the definitive host, the merozoites are released in the intestine, where they develop into male and female gametes.
- After fertilization, the zygote develops into an oocyst containing 2 sporocysts, each having 4 sporozoites.
- These oocysts are shed in feces and are ingested by intermediate host.
- In the intermediate hosts, the sporozoite invade the bowel wall and reach the vascular endothelial walls, where they undergo schizogony producing **merozoites**(**tachyzoites**).
- These spread to muscle fibers and develop into sarcocysts.
- Cow is the *intermediate host* for *S. hominis*. Human infection is acquired by eating raw or undercooked beef. Oocysts are shed in human feces, which contaminate grass الحشائش and fodder العلف eaten by cows.
- In the case of *S. suihominis*, the pig is the intermediate host and human infection is obtained through eating contaminated pork. Human infection with *S. hominis* and *S. suihominis* is related to food habits عادات الطعام.
- Humans are the intermediate host in *S. lindemanni*; the definitive host of which is not yet known. It is believed that *S. lindemanni* may not be a single species but a group of, as yet unidentified species. Humans apparently يوضح get infected by ingestion of oocysts. Sarcocysts develop in the human skeletal muscles and myocardium.

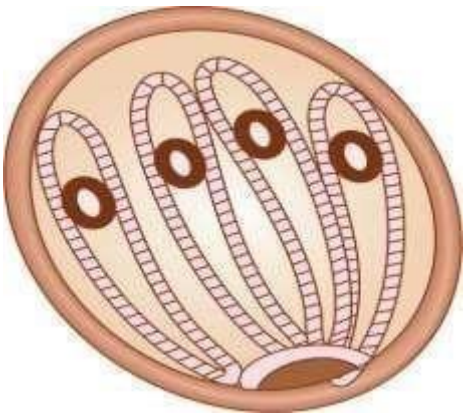
Clinical Features

Intestinal sarcocystosis is usually asymptomatic. Patients may have nausea, abdominal pain, and diarrhea.

Muscular sarcocystosis is also usually asymptomatic but may cause muscle pain, weakness, or myositis, depending on the size of the cyst.



Sarcocyst



Oocyst of *Sarcocystis hominis*

Laboratory Diagnosis

Stool Examination

Characteristically sporocysts or occasionally oocysts can be demonstrated in feces of human beings. Species identification is not possible with microscopy.

Muscular Sarcocystosis

Diagnosis can be made by demonstration of sarcocysts in the skeletal muscle and cardiac muscle by biopsy or during autopsy تشريح الجثة.