

Medical protozoology:

Apicomplexa

A note on taxonomy

Taxonomy is the science of defining groups of biological organisms on the basis of shared characteristics and giving names to those groups.

Practical application
Understanding evolution of life

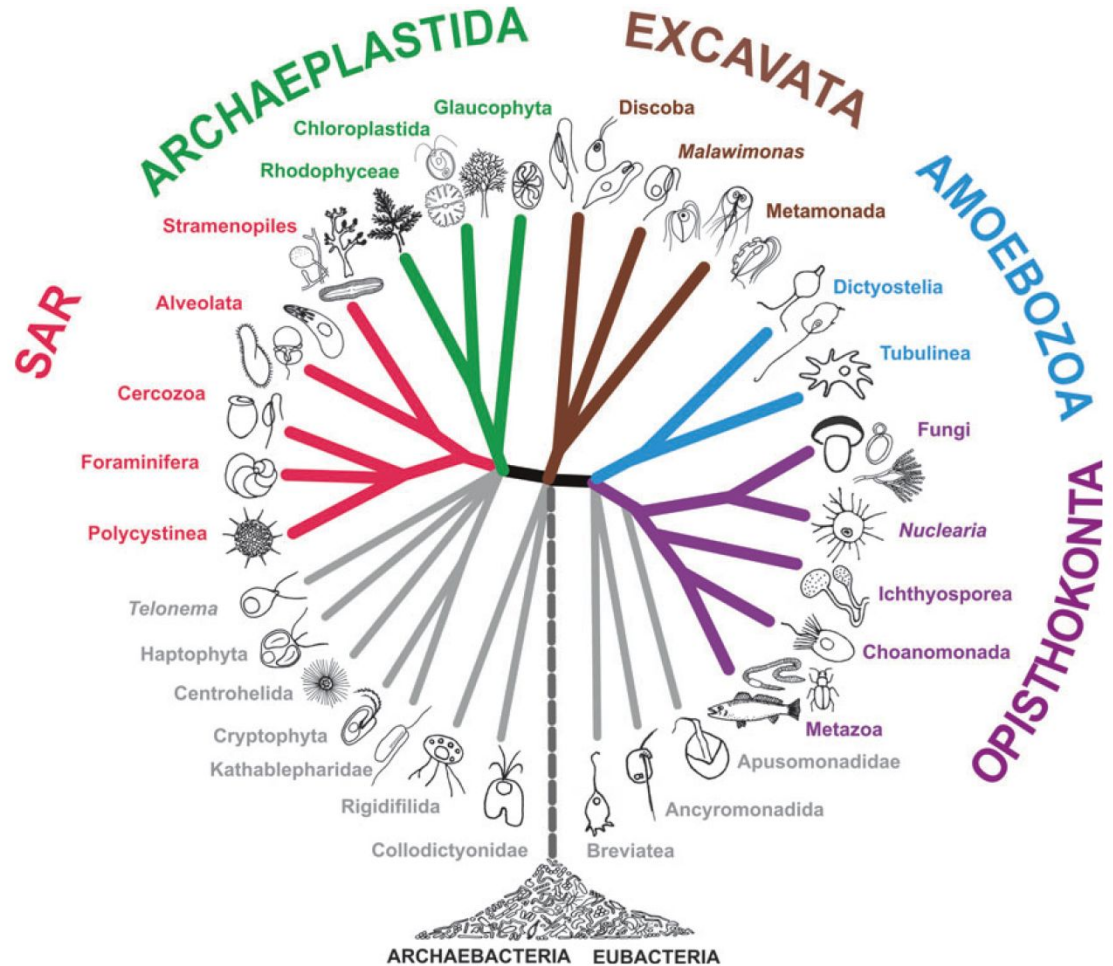


Fig. 1. A view of eukaryote phylogeny reflecting the classification presented herein.

Adl et al. 2012 The revised classification of eukaryotes. The Journal of Eukaryotic Microbiology. 59(2), 429 – 493.

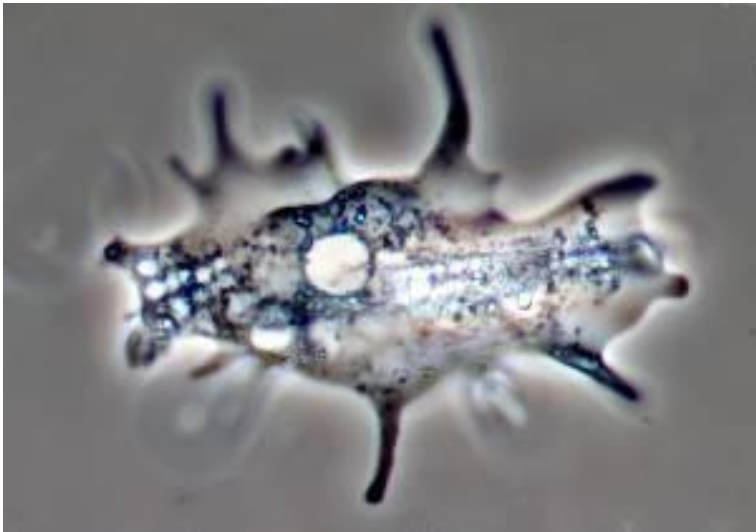
Morphology-based taxonomy

- Depending on the locomotion mode protozoa divided into four major groups:
 - Phylum Flagellates (move by means of flagella)



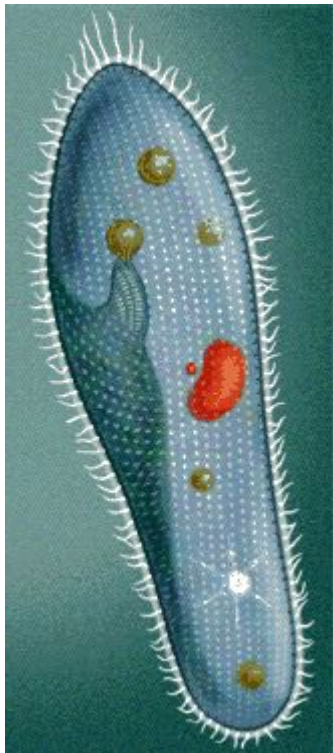
Morphology-based taxonomy

- Depending on the locomotion mode protozoa divided into four major groups:
 - Phylum Amoebae (by pseudopodia)



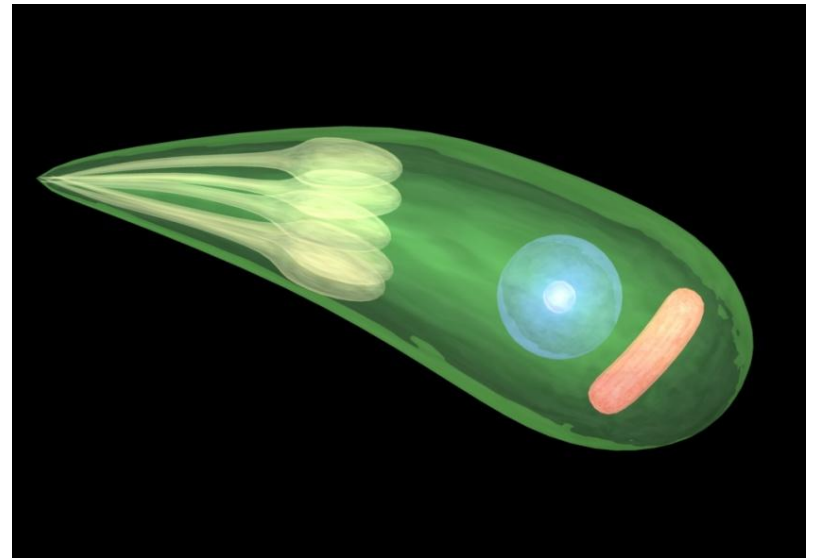
Morphology-based taxonomy

- Depending on the locomotion mode protozoa divided into four major groups:
 - Phylum Ciliates (by cilia)



Morphology-based taxonomy

- Depending on the locomotion mode protozoa divided into four major groups:
 - Phylum Sporozoa (lacking any obvious means of locomotion)



Parasitic Apicomplexa (former Sporozoa)

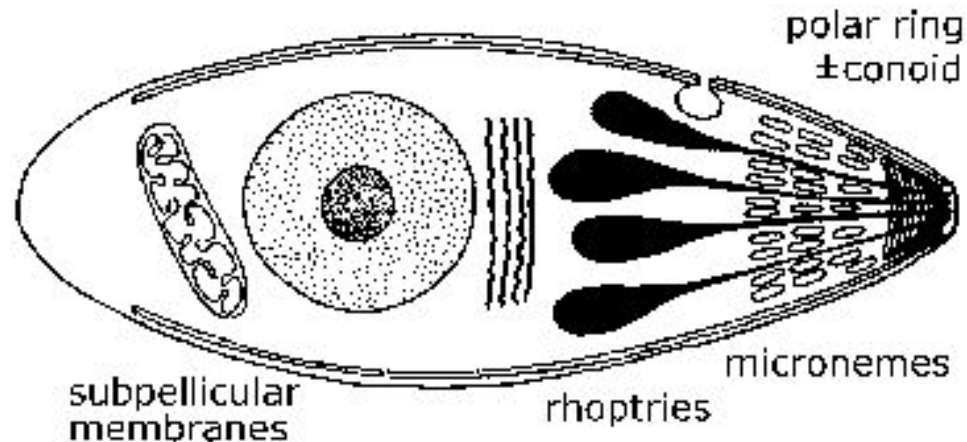
- The apicomplexa are a monophyletic group composed almost entirely of parasitic (ie, no free-living) species.
- Apicomplexa, along with ciliates and dinoflagellates, form a higher order group known as **Alveolata**.
- A major defining characteristic of this group are flattened vesicle-like structures, called **cortical alveolae**, which are found just underneath the plasma membrane.
- Formerly the apicomplexa were part of a group called **Sporozoa** and this name is still sometimes used.

Parasitic Apicomplexa

- A defining characteristic of the apicomplexa is a group of organelles found at one end--called the apical end--of the organism.
- This is a type of plastid called an **apicoplast**, and **anapical complex** structure involved in penetrating a host's cell.

Parasitic Apicomplexa

- This 'apical complex' includes secretory organelles known as **micronemes** and **rhoptries**, **polar rings** composed of microtubules, and in some species a **conoid** which lies within the polar rings.



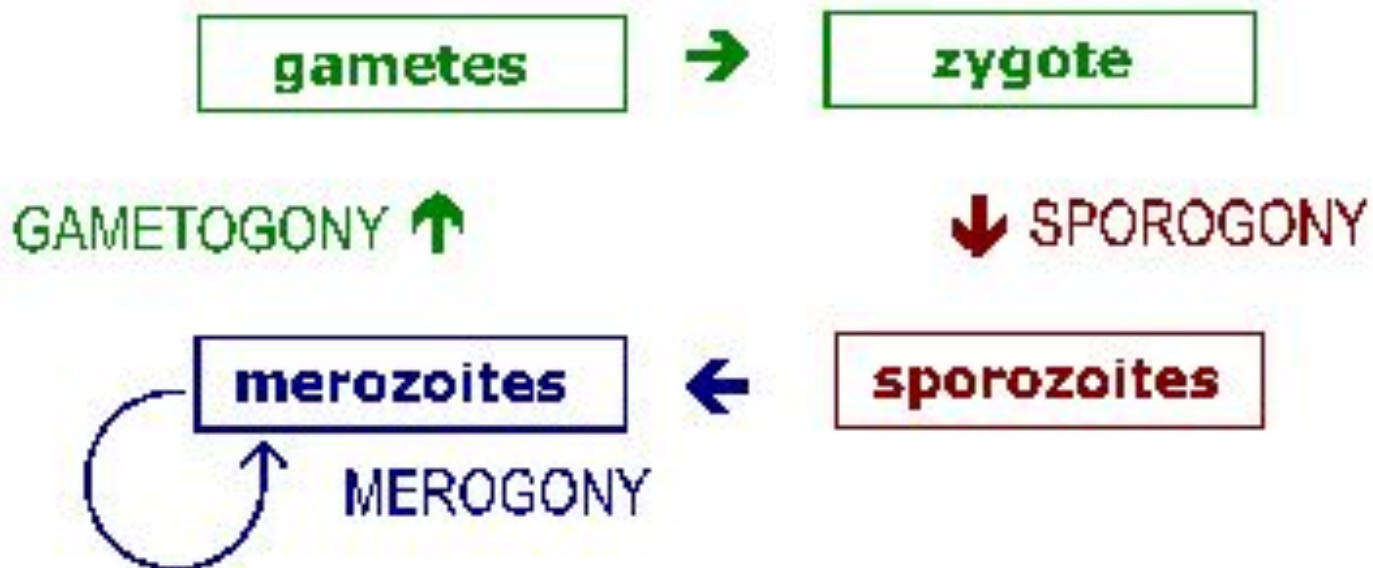
Parasitic Apicomplexa

- At some point during their life cycle, members of the apicomplexa either invade or attach to host cells.
- It is during this invasive (and/or motile) stage that these apical organelles are expressed as well as the subpellicular membranes, which are actually cortical alveoli.
- The apical organelles play a role in interaction of the parasite with the host cell and the subsequent invasion of the host cell.
- Motile forms of apicomplexa crawl along the substratum in a non-ameboid fashion known as gliding motility. Many apicomplexan species have flagellated gametes.

Parasitic Apicomplexa

- The apicomplexa have complex life cycles that are characterized by three distinct processes: sporogony, merogony and gametogony .

General Apicomplexan Life Cycle



Parasitic Apicomplexa

- Although most apicomplexa exhibit this overall general **life cycle** the details can **vary between species**.
- Furthermore, the **terminology** used to describe these various life cycle stages **vary between the species**.
- The life cycle consists of both **asexually reproducing** forms and **sexual stages**.

Parasitic Apicomplexa

- In **monoxenous species** all three of these processes will be carried out in a single host and often in a single cell type or tissue.
- Whereas, in **heteroxenous species** the various processes will be carried out in different hosts and generally involve different tissues.

Parasitic Apicomplexa

- **Sporogony** occurs immediately after a sexual phase and consists of an asexual reproduction that culminates in the production of sporozoites.
- **Sporozoites** are an invasive form that will invade cells and develop into forms that undergo another asexual replication known as merogony.

Parasitic Apicomplexa

- **Merogony** and the resulting **merozoites** are known by many different names depending of the species. In contrast to sporogony, in which there is generally only one round of replication, quite often there are **multiple rounds of merogony**.
- In other words, the merozoites, which are also invasive forms, can **reinvade cells** and initiate another round of merogony.

Parasitic Apicomplexa

- As an alternative to asexual replication merozoites can **develop into gametes** through a process variously called gametogony, gamogony or gametogenesis.
- As in other types of sexual reproduction, the gametes fuse to form a **zygote** which will undergo sporogony.

Parasitic Apicomplexa

- The apicomplexa are an extremely large and diverse group (>5000 named species). Seven species infect humans.
- *Plasmodium*
- *Babesia*
- *Cryptosporidium*
- *Isospora*
- *Cyclospora*
- *Sarcocystis*
- *Toxoplasma*

Parasitic Apicomplexa

- *Plasmodium*, as the causative agent of **malaria**, has the greatest impact on human health.
- *Babesia* is a relatively rare **zoonotic infection**.
- The other five species are all classified as **coccidia**. However, recent molecular data indicates that *Cryptosporidium* is more closely related to the **gregarines** than to the coccidia. The coccidia are generally considered **opportunistic pathogens** and are often associated with AIDS.

Parasitic Apicomplexa

- Several apicomplexan parasites are also important in terms of **veterinary medicine** and **agriculture**. Most notable are *Babesia* and *Theileria* in cattle and *Eimeria* in poultry.

Parasitic Apicomplexa

- Diseases caused by apicomplexan organisms include, but are not limited to:
 - Forms of coccidiosis including:
 - Cryptosporidiosis (*Cryptosporidium parvum*)
 - Cyclosporiasis (*Cyclospora cayetanensis*)
 - Isosporiasis (*Isospora belli*)
 - Toxoplasmosis (*Toxoplasma gondii*)
 - Malaria (*Plasmodium*)
 - Babesiosis (*Babesia*)

Intestinal Sporozoa

1- *Cryptosporidium* spp.

The species of medical importance to man are:

Cryptosporidium parvum of cattle.

Cryptosporidium muris of rodents.

Disease: Cryptosporidiosis.

Distribution: Worldwide.

Definitive hosts: Man.

Reservoir host: according to species;
rodents, cattle, birds, reptiles & fish.

Site: Within the brush border of the villi
between membrane & cytoplasm.

Morphology

Oocyst: is spherical in shape, measures 4-6 μm in diameter & contains 4 sporozoites.

Types of oocysts

- a) Thin wall oocyst.
- b) Thick wall oocyst.

Infective stage

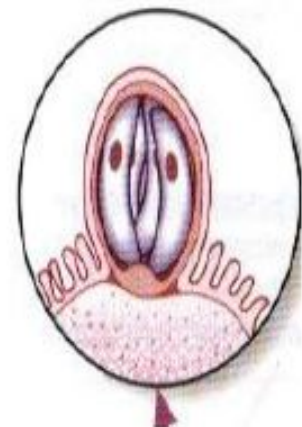
Mature sporulated oocysts
[thin & thick wall].

Sporulation: Immediately mature in feces.

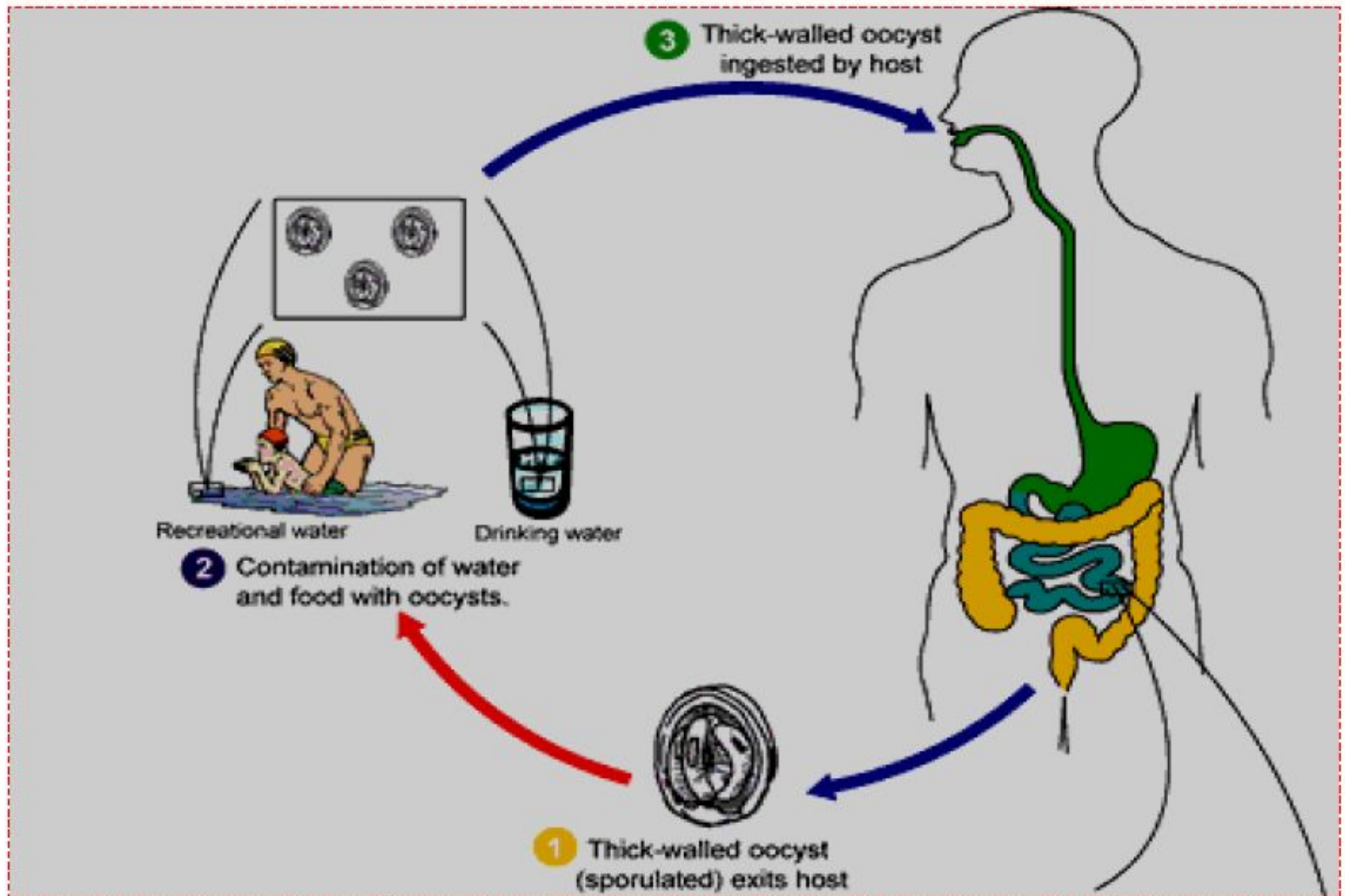
Thin-walled oocyst
(sporulated)



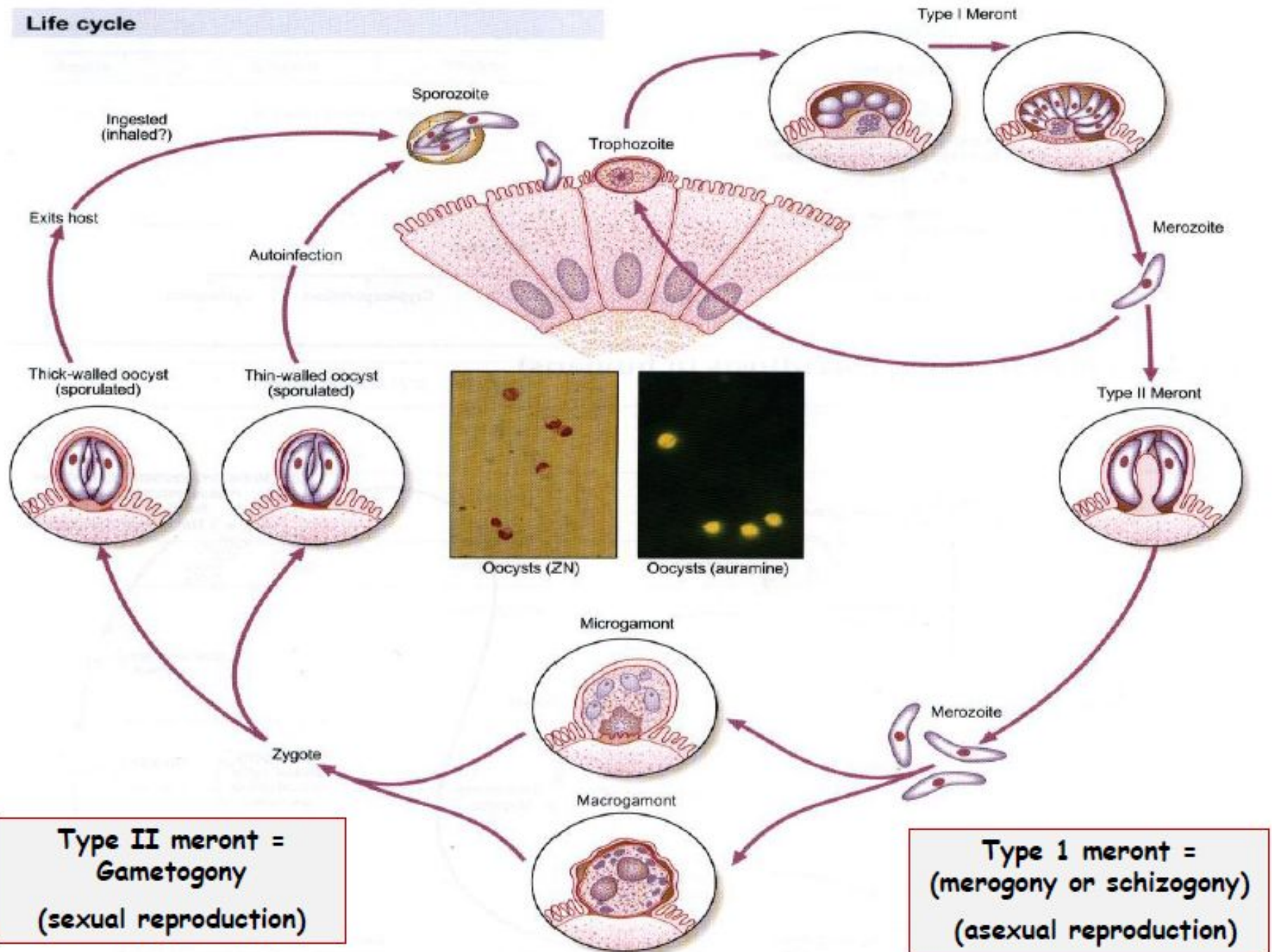
Thick-walled oocyst
(sporulated)



Life cycle of *Cryptosporidium* spp.



Life cycle



Mode & sources of infection with *Cryptosporidium* spp.

Human infection is usually waterborne.

Mode of infection

Ingestion of mature oocysts.

Sources of infection

1. Contamination of food or drinks.
2. Direct infection from carriers.
3. **Autoinfection:**
 - External** via thick oocysts.
 - Internal** via thin oocysts.
4. Mechanical transmission by house flies.



Thick-walled



Thin-walled

4-6 μ

Pathogenesis of cryptosporidiosis

Infection is accompanied with changes in the structure and function of the intestine in the form of:

Atrophy of intestinal villi, lengthening of the crypts
&
infiltration of lamina propria by inflammatory cells.

This results in decreased ability of the intestine to absorb proper amounts of water & nutrients leading to dehydration and anorexia.

Clinical picture of cryptosporidiosis

Cryptosporidium is an opportunistic parasite commonly infecting persons with immunodeficiency

such as those with:

- a) acquired immune deficiency syndrome AIDS.
- b) hypogamma globulinemia.
- c) chemotherapy or radiotherapy.

I- Infection in immuno-competent subjects *results in:*

- a) Asymptomatic cases.**
- b) Mild self limited diarrhoea [watery, non-bloody] for 2 weeks.**
- c) In children, it is accompanied by fever, abdominal discomfort, anorexia, nausea & loss of weight.**

II- Infection in immunocompromized patients *can cause:*

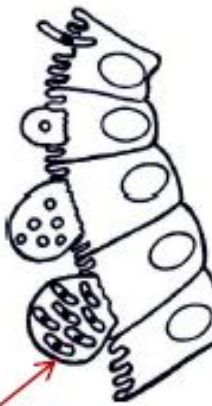
- a) acute fatal diarrhea ,vomiting, severe abdominal pain, fever & dehydration and**
- b) dissemination of the parasite to other organs causing → respiratory infection, hepatitis, cholecystitis & pancreatitis.**

Diagnosis of cryptosporidiosis

1. Clinically.
2. Laboratory.

Laboratory diagnosis of cryptosporidiosis

- 1] Stool examination
 - a] Macroscopically: Stool is loose and watery.
 - b] Microscopically: to detect oocysts by:
 - * Direct smear.
 - **Concentration methods [Sheather's sugar floatation].
- 2] Small intestinal biopsy: shows the developmental stages of the parasite [meronts & gamonts] .
- 3] Detection of *Cryptosporidium* antigen in stool.



Meront



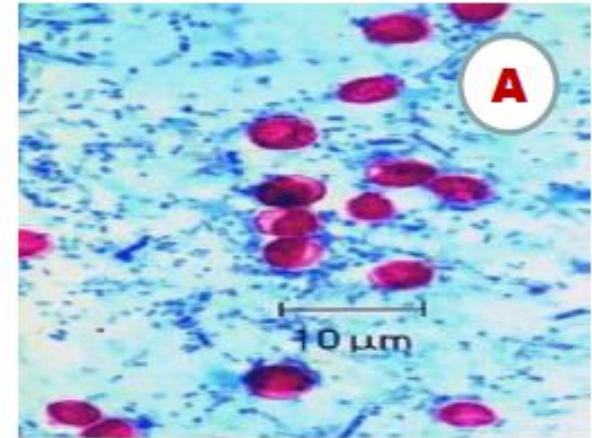
Gamont

Oocysts can be visualized by

* Staining with acid-fast stain

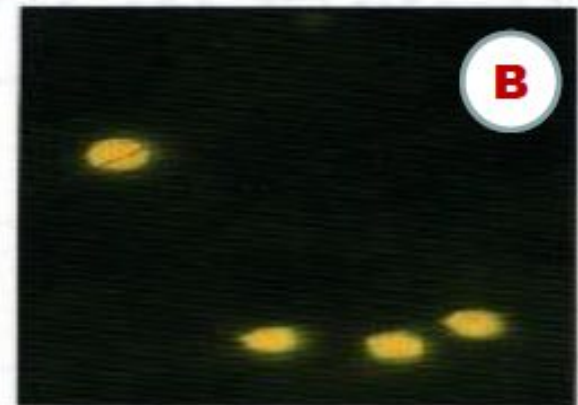
(Modified Ziehl Neelsen stain)

The oocysts appear as bright red or pink, oval or round bodies against a green background, (A).



* Immunofluorescence assay

by which fluorescent oocysts can be easily detected, (B).



Oocysts (auramine)

Treatment of cryptosporidiosis

- 1- It is a self limited disease in immunocompetent patients.
- 2- In immunocompromized patients:
 Spiramycin (Rovamycin):
 3 gm/day orally in divided dose for 2-4 weeks.
- 3- Fluid therapy: To maintain fluid & electrolyte balance.

Prevention and control

Cryptosporidiosis is a zoonotic disease & may be acquired from domestic animals.

Oocysts are highly resistant to chemicals used to treat drinking water.

- 1- Avoid contamination of food & drink with human and animal excreta.**
- 2- Proper filtration & boiling of drinking water.**
- 3- Insect control.**

Intestinal Sporozoa

2- *Cyclospora cayetanensis*

Disease: Cyclosporiasis.

Distribution: Worldwide.

D.H.: Man.

Habitat: Enterocytes of small intestine.

Mode of infection

Ingestion of sporulated oocysts with food or drink.

Sporulation: Needs about 5-10 days outside.



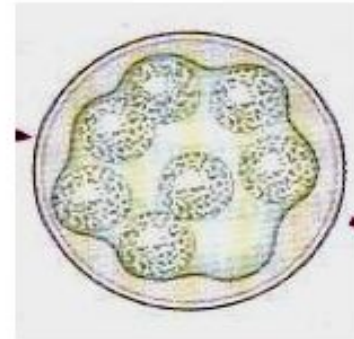
8-10 μ

No autoinfection takes place in cyclosporiasis.

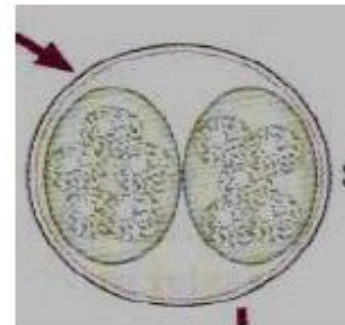
Morphology

a. Unsporulated oocyst

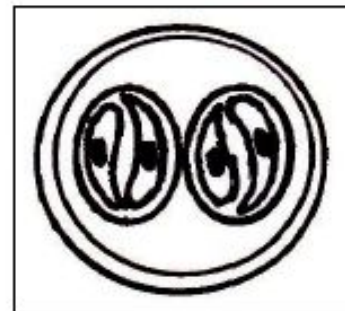
It is spherical in shape, 8-10 μm in diameter with a central morula. It contains 6-9 retractile spheres. It transforms into developing oocyst [*with 2 sporocysts*] then sporulated oocyst .



Unsporulated oocyst



Developing oocyst

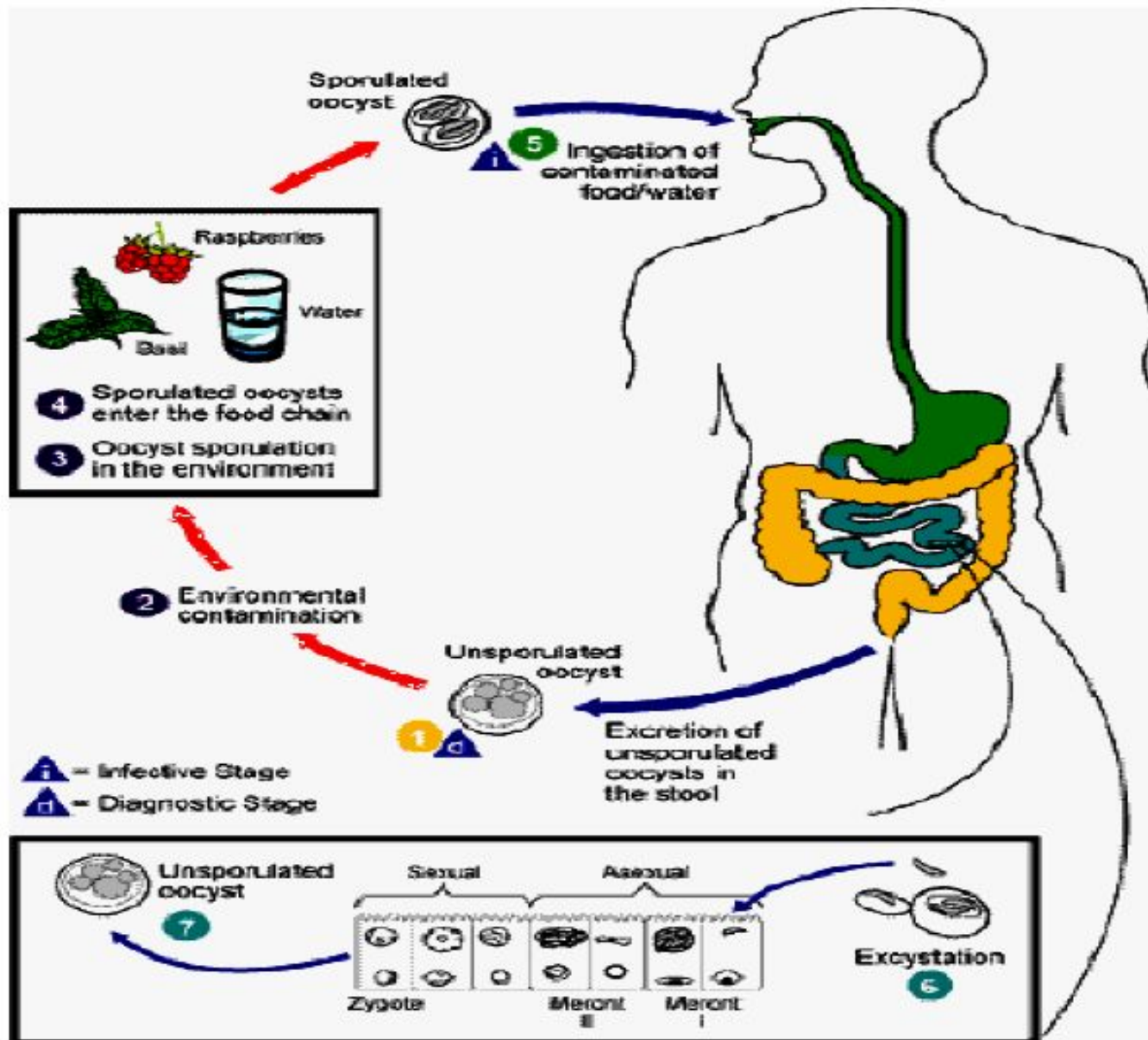


Sporulated oocyst

b. Sporulated oocyst (mature oocyst) (infective stage)

It contains 2 sporocysts, each contains 2 sporozoites.

Life cycle of *Cyclospora cayetanensis*



Pathogenesis & Clinical picture

Inflammatory changes develop in the affected area of the small intestine in the form of villous atrophy & crypt hyperplasia .

Immunocompetent patients suffer from:

Acute watery diarrhea [prolonged & relapsed], nausea, vomiting, flatulence and colicky abdominal pain.

In immunocompromized individuals

(opportunistic infection):

The disease is severe, prolonged & recurrent

and

Biliary affection may develop.

Diagnosis of cyclosporiasis

1 - Clinically.

2 - Laboratory:

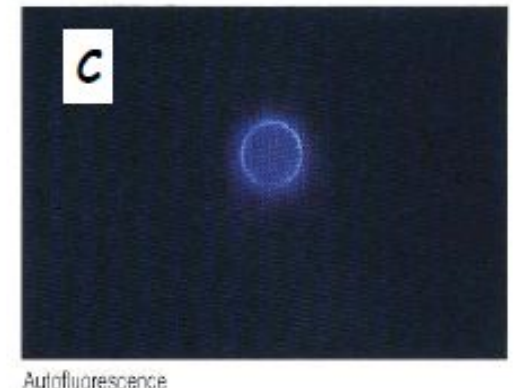
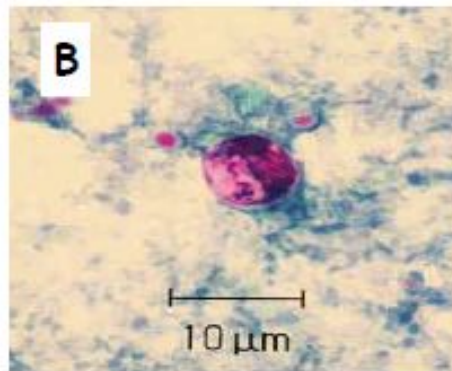
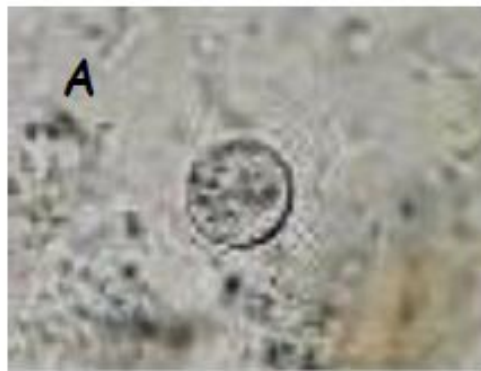
Laboratory diagnosis is done by stool examination for detection of oocysts
By

*Direct smear.

**Concentration methods: [as Sheather's sugar floatation].

The specimen can be examined
either

Fresh unstained [A] and
stained with acid-fast stain [B] or fluorescence [C].



Treatment

- 1 - Combination of:
Trimethoprim (160 mg) and
Sulfamethoxazole (800 mg) twice daily for 7 days.
- 2 - Fluid therapy:
To maintain fluid & electrolyte balance.

Prevention and control

- 1 - Treatment of cases.
- 2 - Proper washing of vegetables & fruits.
- 3 - Boiling of drinking water.

Intestinal Sporozoa

3- *Isospora belli*

Disease: Isosporiasis.

Distribution: Worldwide.

D.H.: Man .

Habitat: Enterocytes of small intestine.

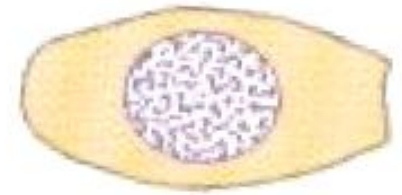
Mode of infection

Ingestion of sporulated oocysts with food or drink.

Sporulation: Occurs both within the host & outside human body (needs 48 hours).

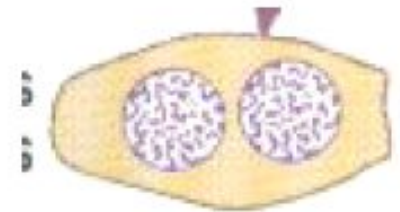
Morphology

a. Unsporulated oocysts: Spindle shape with blunt ends, translucent, $20\text{-}30 \times 10\text{-}12\text{ }\mu\text{m}$ in size and contain spherical mass of protoplasm.



Unsporulated oocyst

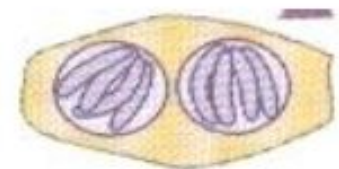
b. Sporulated oocysts
(mature oocysts)
Contain 2 sporocysts each contains
4 sporozoites.



Developing oocyst

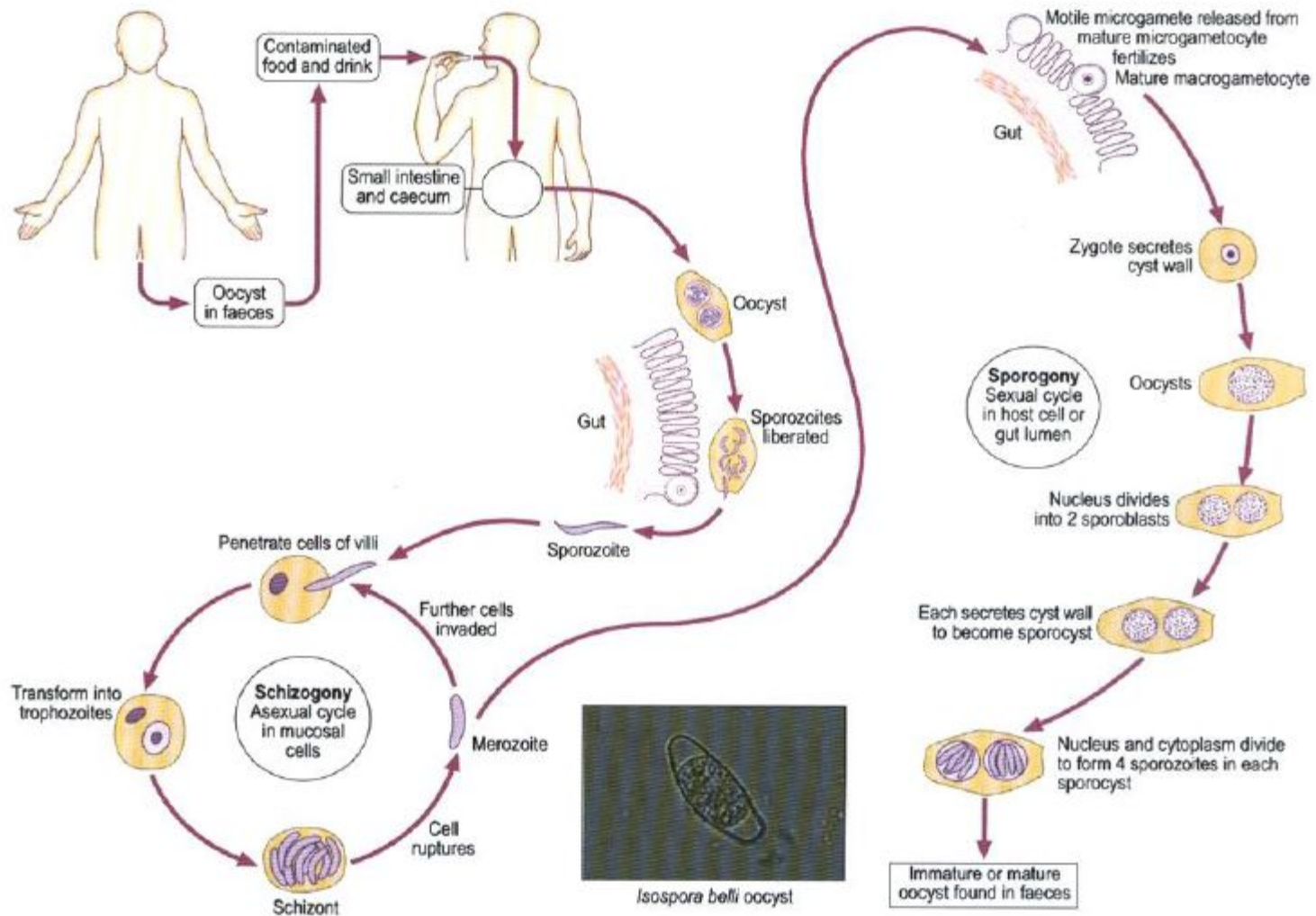
Mode of infection

Ingestion of food or drink
contaminated with sporulated
oocysts



Sporulated oocyst

Life cycle of *Isospora*



Pathogenesis & Clinical picture of isosporiasis

Inflammatory changes develop in the affected intestinal epithelium.

The infection in immunocompetent individuals is often asymptomatic or produces self-limited diarrhea lasting for 2-3 weeks with colic, distension and low grade fever.

The infection in immunocompromized individuals (opportunistic infection) takes the form of severe diarrhea, malabsorption, dehydration, loss of weight and may be even fatal.

Diagnosis of isosporiasis

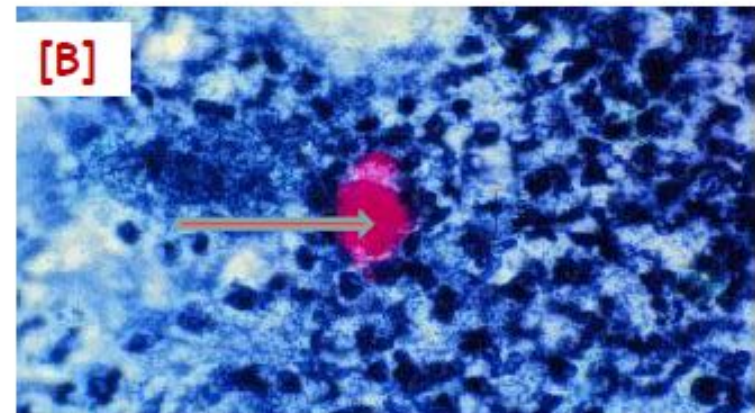
1- Clinically: Clinical picture.

2- Laboratory: Stool examination for detection of oocysts by:

* Direct smear.

**Concentration methods [Sheather's sugar floatation] .

Oocysts appear transparent in fresh unstained specimens [A],
can be found better after staining by iodine and acid-fast
stain [B]



Treatment

1 - Combination of Trimethoprim (160 mg) and Sulfamethoxazole (800 mg) orally every 6 hours for 10 days followed by the same dose twice daily for 3 weeks.

2 - Fluid therapy

To maintain fluid & electrolyte balance.

Prevention and control

1- Treatment of cases and carriers.

2- Avoid contamination of food & drink with human excreta.

3- Insect control and protection of food.