

University of Zambia

School of Medicine

Apicomplexa Parasites

Cryptosporidium and Isospora

3rd /4th Year MBChB

2023

Phylum **APICOMPLEXA**

Class **SPOROZOEA**

Subclass **COCCIDA**

Order **EUCOCCIDIIDA**

Suborder **EIMERIINA**

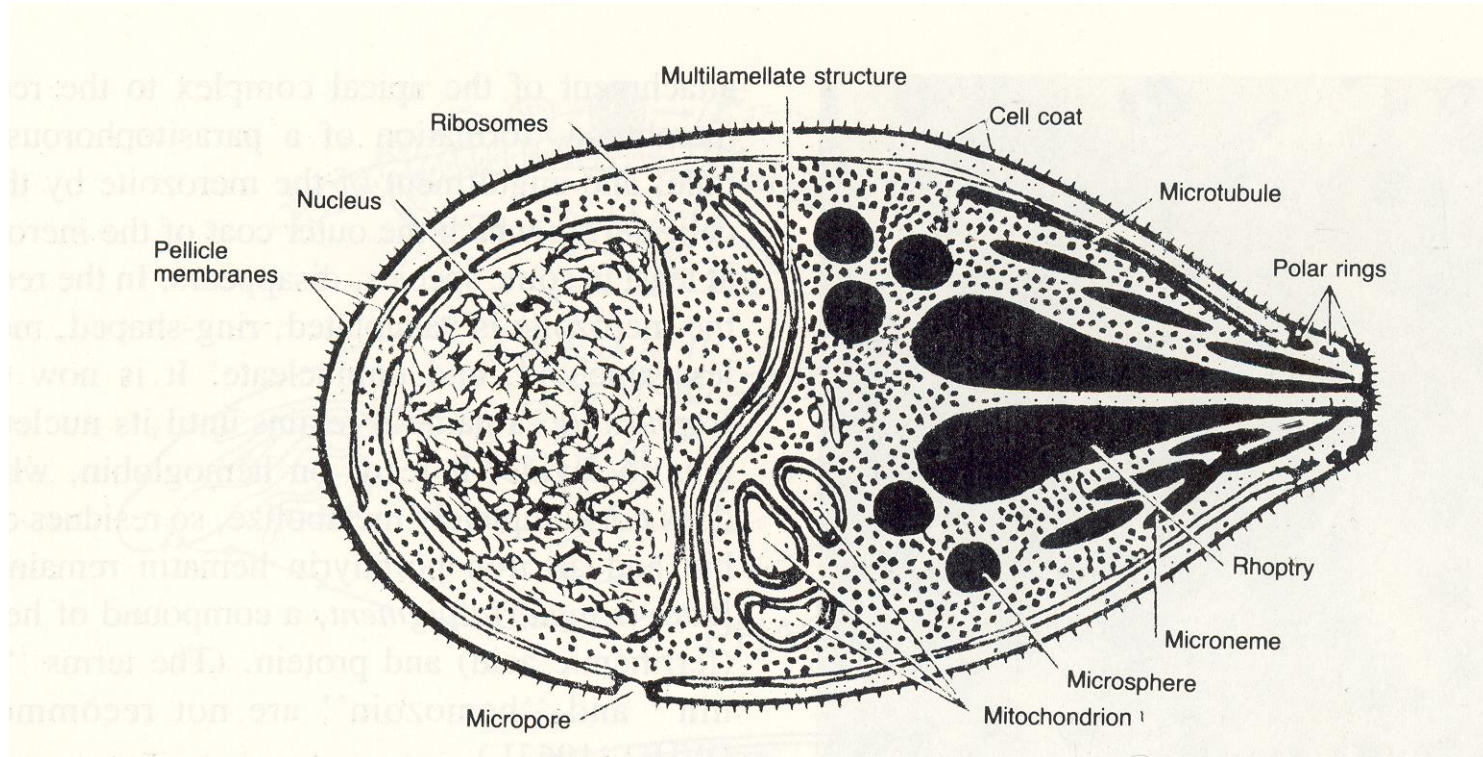
Genus **Cryptosporidium,**

Pneumocystis, Sarcocystis, Toxoplasma, Isospora

Suborder **HAEMOSPORINA**

Genus **Plasmodium**

Plasmodium. Diagram of a merozoite showing the principal features seen in electron micrographs of longitudinal section.



Cryptosporidiosis

etiologic agent Cryptosporidium parvum or C. hominis

Habitat:

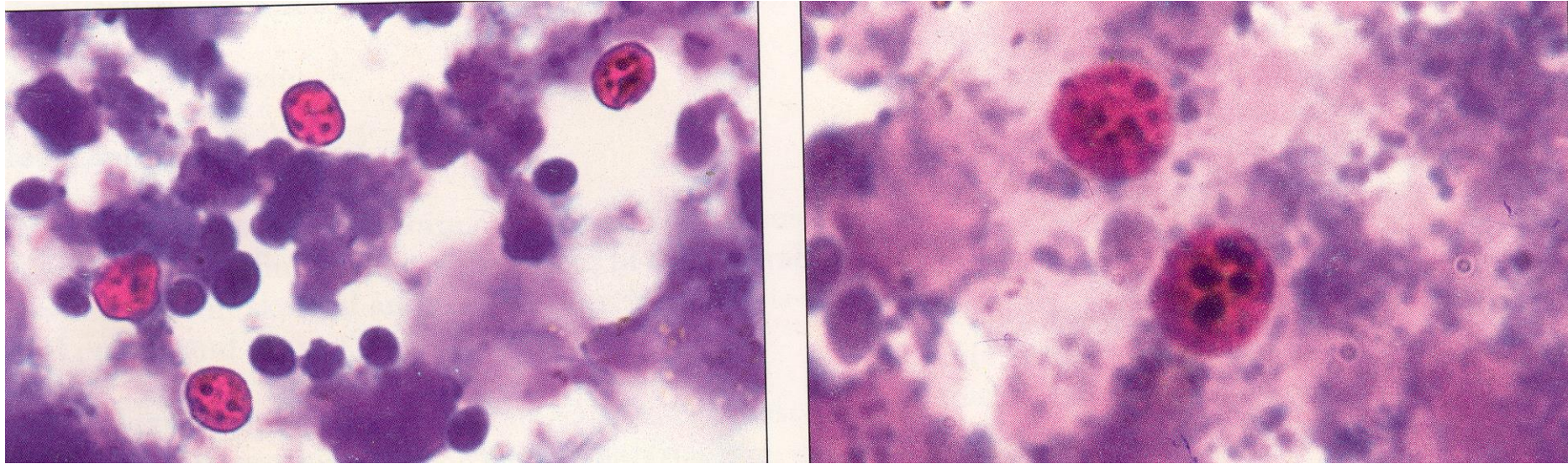
- Brush borders of mucosal epithelium
- Stomach
- Crypts of L.I and villi of the S.I
- Rectum
- Appendix
- Pulmonary tree

Species, host and zoonotic potential

No.	Species	Usual host	Zoonotic potential
1	<i>C. muris</i>	Mouse, cattle and gazelles	Yes
2	<i>C. parvum</i>	Mouse sheep, gazzelle, buffalo, cat, humans	Yes
3	<i>C. meleagridis</i>	Turkey	Yes
4	<i>C. wrairi</i>	Guinea pig	No
5	<i>C. felis</i>	Cats	Yes
6	<i>C. serpentis</i>	Snakes	No
7	<i>C. baileyi</i>	Chicken	No
8	<i>C. saurophilum</i>	Skink	No
9	<i>C. andersoni</i>	Cattle	Yes
10	<i>C. canis</i>	Dog	Yes
11	<i>C. hominis</i>	Humans	Yes

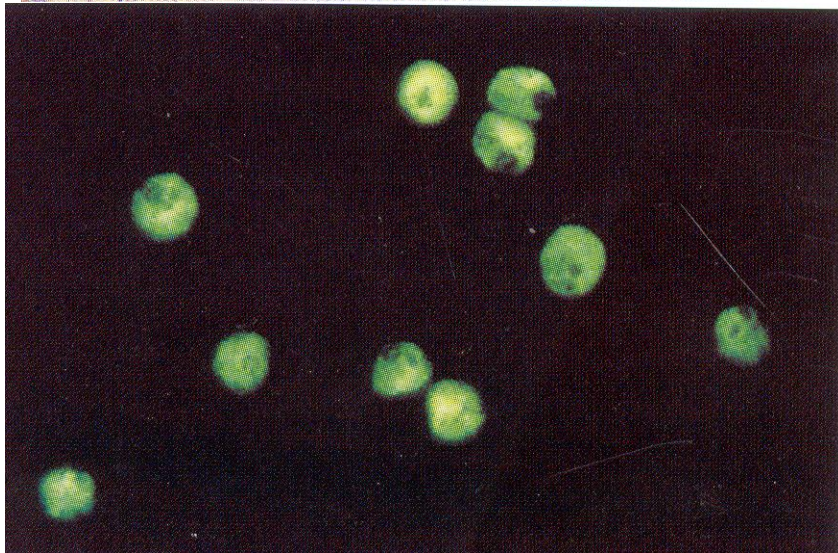
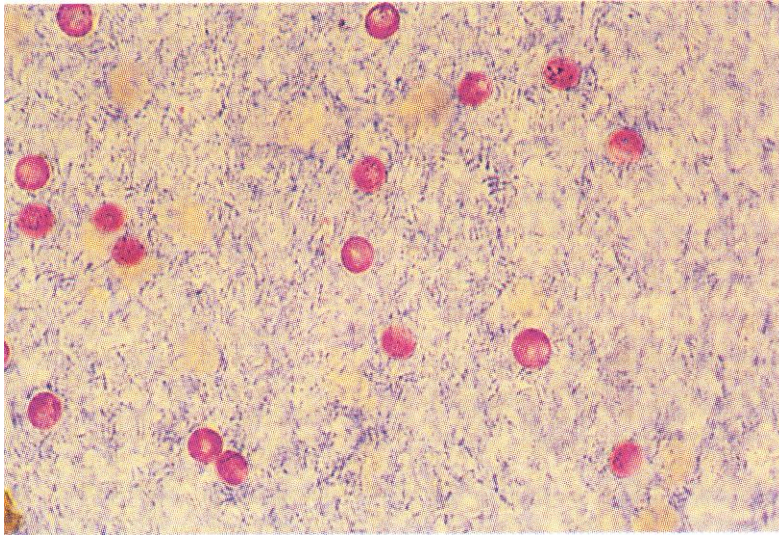
Morphology

- Oocyst with four sporozoites



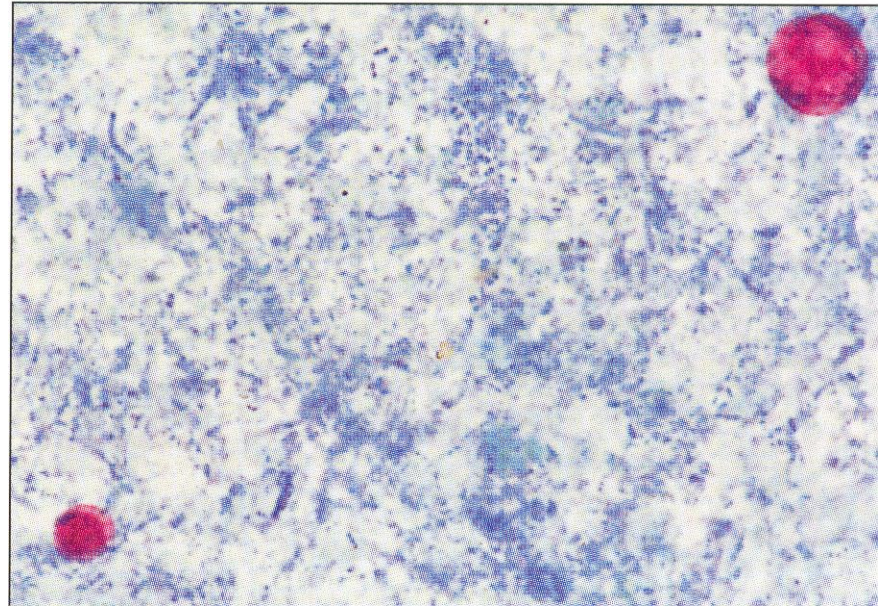
In these acid-fast stained fecal smears, oocysts of *C. parvum* stain an intense red and the granules are characteristically black. In acid-fast stained preparations, **yeasts**, which can be mistaken for *Cryptosporidium* oocysts, take a **green stain**. Various modifications of acid-fast stains can be used and the oocysts of *Cryptosporidium* may range from light pink to intense red.

Cryptosporidium. Oocyst in faeces,
Zeil-Neelsen stain

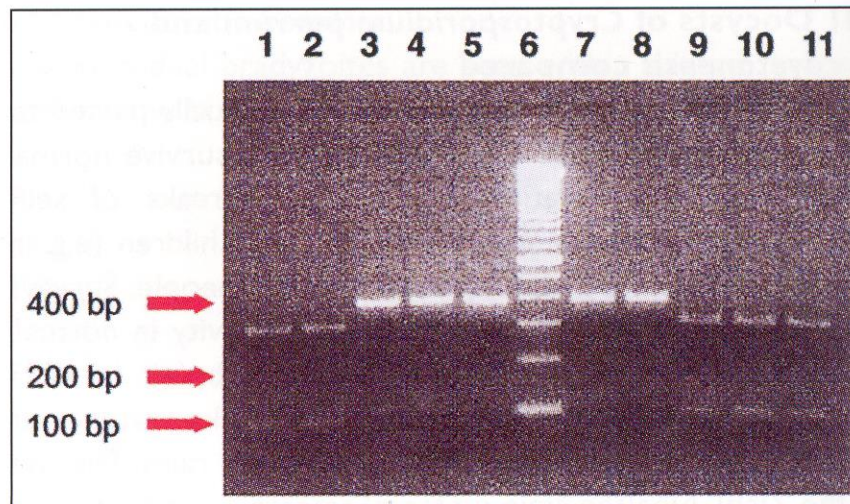


Auramine stain for oocysts of
Cryptosporidium parvum.
Fluorescent staining of oocysts
with auramine

Oocysts of *Cryptosporidium parvum* and *C. cayetanensis* compared .
C. parvum is enzootic in young calves. It is usually passed to humans in water containing oocysts, which survive normal chlorination and filtration. Over 50% in one outbreak. In 1993 over 400 000 people had diarrhoea when lake water near Milwaukee became contaminated after heavy rains. Both parasites stain red with Ziehl-Neelsen stain. However, *C. cayetanensis* typically takes up variable amounts of the stain and the oocysts are larger (8-10 μm , compared with 4-6 μm).



Differentiation of genotypes of *Cryptosporidium parvum* by polymerase chain reaction (PCR) Genotyping by the PCR-restriction fragment linked polymorphism (RFLP) technique of isolates from human and animal faeces has revealed at least eight genotype: Genotype I, only been found in humans, 1/3: genotype 2, 2/3, rare infections were caused by genotype 3.



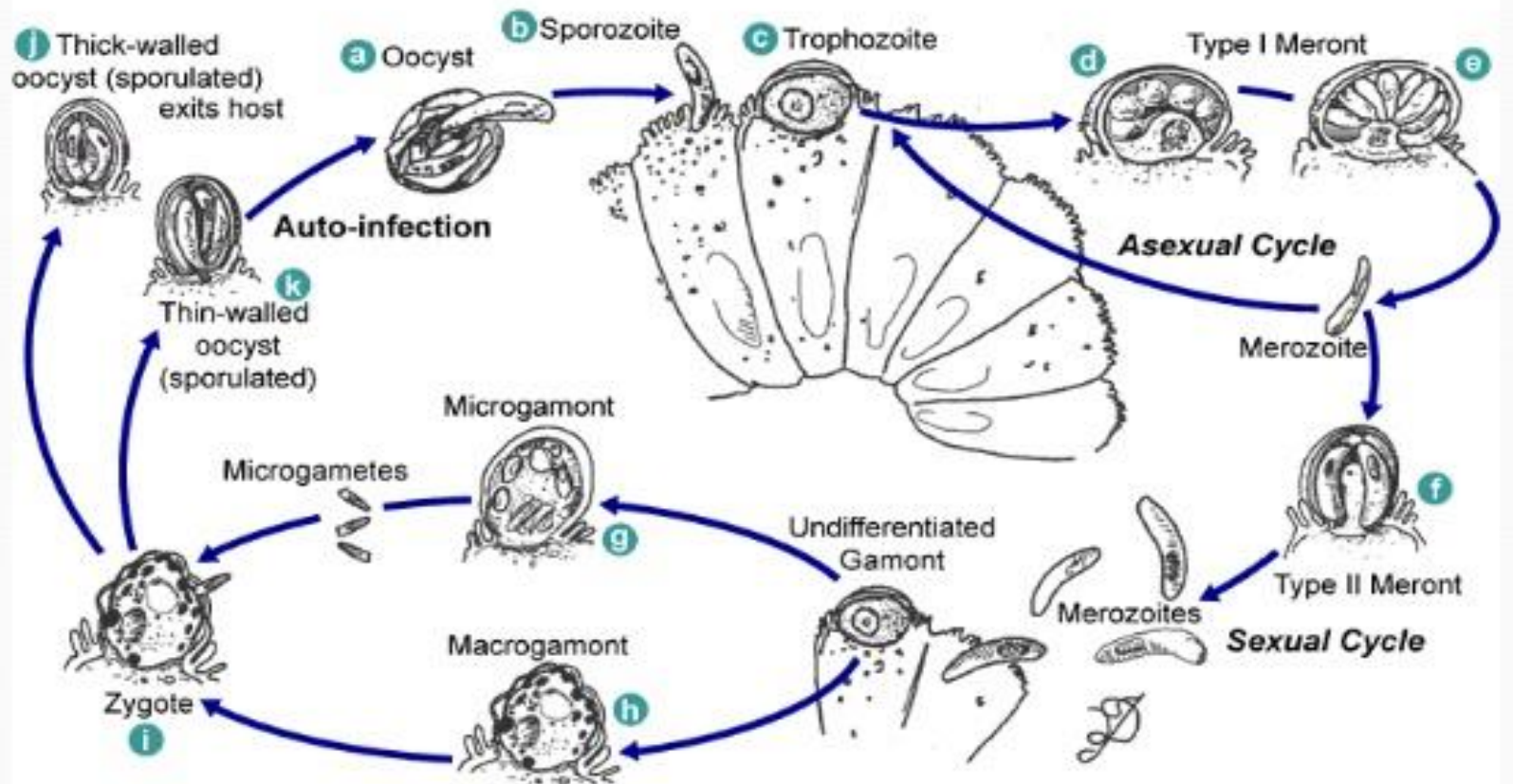
Transmission

- Infections are acquired by ingesting oocysts transmitted by faecal-oral route
 - Ingestion of contaminated food and water
- Autoinfection
- Infective form - oocyst (with four sporozoites)

Life Cycle

- Monoxenous
- Suitable host – man
- Reservoirs – man, cattle, cat, dog

Life Cycle



<http://www.dpd.cdc.gov/dpdx/HTML/Cryptosporidiosis.asp?body=Frames/A-F/Cryptosporidiosis>

Life Cycle

- Ingested oocysts reach small intestines or respiratory system
- Four sporozoites released, adhere to surface mucous lying above host epithelial cell's apical membrane
- Release enzymes, break down mucous that enables sporozoites make contact with & bind to host cell membrane through receptor-ligand interactions

Life Cycle

- Sporozoites discharge contents of their apical organelles, including chemicals
- These induce host cell membrane form protrusions which surround the parasites to become encapsulated within a parasitophorous vacuole (pv)
- Membrane that ultimately surrounds the pv contains both parasite & host cell components

Life Cycle

- Pv sits just inside the host cell apical membrane & is separate from the host cytoplasm
- Within the pv, the sporozoites transform into trophozoites and then into meronts (schizonts)
- Divides asexually by merogony to produce type 1 meronts containing 8 merozoites & type 2 meronts containing 4 merozoites

Life Cycle

- Further cycles of merogony might take place or the type 2 meronts may undergo gametogony
- Results into production of microgamonts (male) & macrogamonts (female)
- Microgamonts produce microgametocytes & released to find macrogamonts with which they fuse

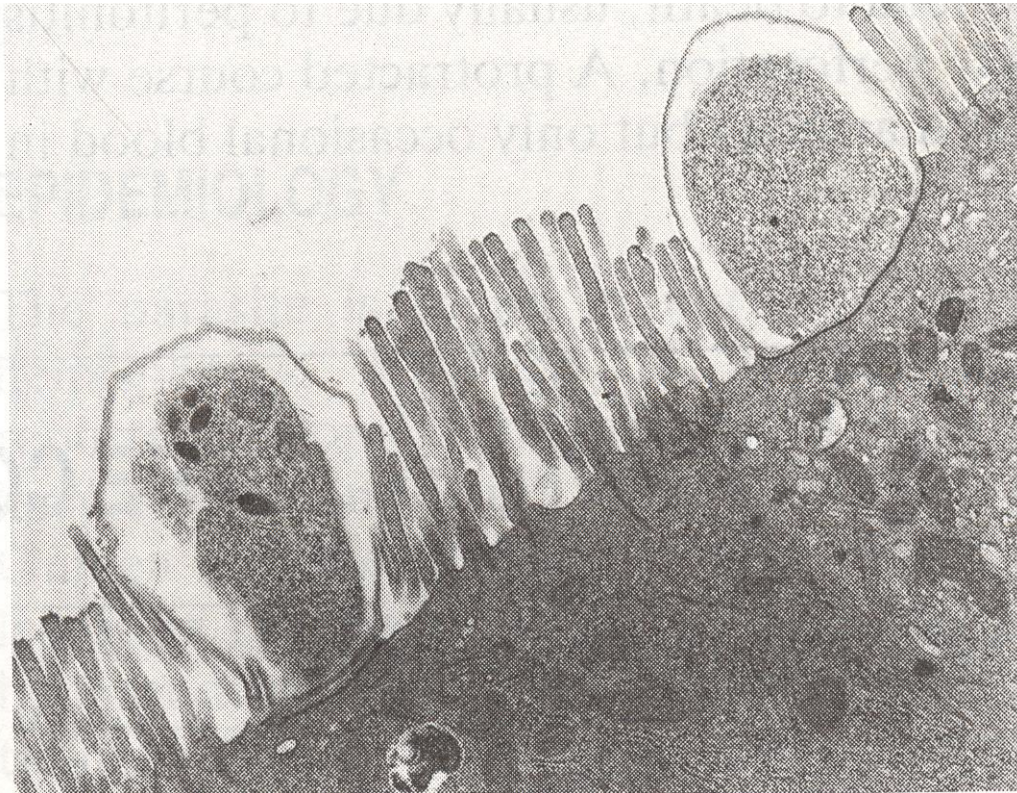
Life Cycle

- Once a macrogamont has been fertilized, it develops into a zygote
- About 20% of zygotes form thin-walled oocysts that are able to reinfect the host
- Other 80% of zygotes develop into thick-walled oocysts shed in faeces into the environment & immediately infectious

Life Cycle

- Can survive for prolonged periods, waiting to be ingested in contaminated food and water

Transmission electron micrograph of *C. parvum* trophozoites in distal duodenal mucosa. The trophozoites occupy an intracellular but extracytoplasmic position.



Ultrastructure of Cryptosporidium In individuals with HIV infection, the existence of large numbers of Cryptosporidium on the surface of the intestinal mucosa can cause intractable, profuse, watery diarrhoea and weight loss. 7% death rate in people with AIDS in Western countries. The organisms in different stages of schizogony are seen surrounded by a membrane of host-cell origin in this electron micrograph, giving the parasites the false appearance of being extracellular. The section is from a biopsy of rectal mucosa from one of the earliest known patients with AIDS. The parasites can colonise any part of the intestine from the pharynx to the rectum.



Pathogenesis of Cryptosporidium

1. Buries into intestinal lining of the gut causing damage to mucosal epithelium
 - Goes through life cycle
2. Alters osmotic pressure
 - Acute diarrhoea: watery, secretory, bloodless, abdominal pains nausea, weight loss

Pathogenesis & Symptoms

3. Atrophy of intestinal villi

- Alters uptake of fluids, electrolytes & nutrients, malabsorption syndrome

4. Asymptomatic or self-limiting, 1-2 weeks duration in the immuno – competent

30 days the immuno-compromised

50 + stools per day, fluid loss, dehydration

5. Ectopic: Bile duct, upper respiratory and lungs

Innate Immune Response

- White blood cells phagocytize parasites
 - Segmented neutrophils
 - Macrophages
 - eosinophils

Cell mediated IR

- CD4+ T cells – early infection
- CD8+ T cells – elimination
- CD154 and CD40 – stimulate nitric oxide, IFN- γ , IL-12, T cell response, apoptosis
- Other cytokines
 - TNF $-\alpha$, IL-1B, IL-2, IL-4, IL-10, IL-15
- Patients with AIDS-decreases CD4+ count

Humoral IR

- IgM
- IgG
- IgA
- X-linked immunodeficiency
- Mutations in CD154 gene
- Defected IgM cannot mount immune response

Diagnosis: Presumptive diagnosis (clinical) and lab examinations

Multiple stool specimens

1. Modified Ziehl-Neelsen stain or Trichrome stain
2. Small bowel biopsies – light microscopy, - haematoxylin, Giemsa, ZN, Tolouidine blue O
3. EM
4. PCR
5. Auramine fluorescent stain
6. EIA
7. Rapid Immunochromatographic cartridge assaya

Treatment of Cryptosporidiasis:

no safe effective chemotherapy

1. Individuals with AIDS

-Anti-retroviral therapy & combination with
Albendazole 400mg twice daily for one month

2. Withdrawal of immunosuppressive therapy

4. Supportive therapy with fluid, electrolytes and nutrients replacement

Epidemiology of Cryptosporidiosis:

1. World wide, ubiquitous
2. Immunocompetent – self-limiting within 30 days, (1-2 weeks)
3. Immunocompromised – chronic
 - a. HIV and AIDS
 - b. Immunosuppressive therapy
 - c. Organ transplant
 - d. Cancer chemo, radiation therapy
 - e. Steroid Tx

- f. Congenital agamma globulinemia
or hypo gammaglobulinemia
 - g. Splenectomised
4. 50% in Haiti,
32% Zambia Lusaka; adult attack
rate – 1.74/Y/adult,
5. HIV positives 81% Cryptosporidiosis

Prevention: as any other fecal/oral route

1. Personal hygiene,
disinfectants, bleach,
formalin
2. Good sanitation

Isospora belli

= Coccidiosis

Habitat: Intestinal mucosa
Intracellular in epithelium of
jejunum/duodenum

Transmission of Isopspora belli

1. Ingestion of Oocyst with two sporocysts
(faecal-oral route)

Morphology

1. Oocyst- oval (**flask-shaped**), 20 - 33 μ
X 10 - 19 μ , one end of oocyst narrower.

Life Cycle

- Ingestion of oocysts containing infective sporozoites
- Sporozoites emerge in the SI & invade epithelial cells
- Transform into trophozoites, schizonts which divide to produce more merozoites

Life Cycle

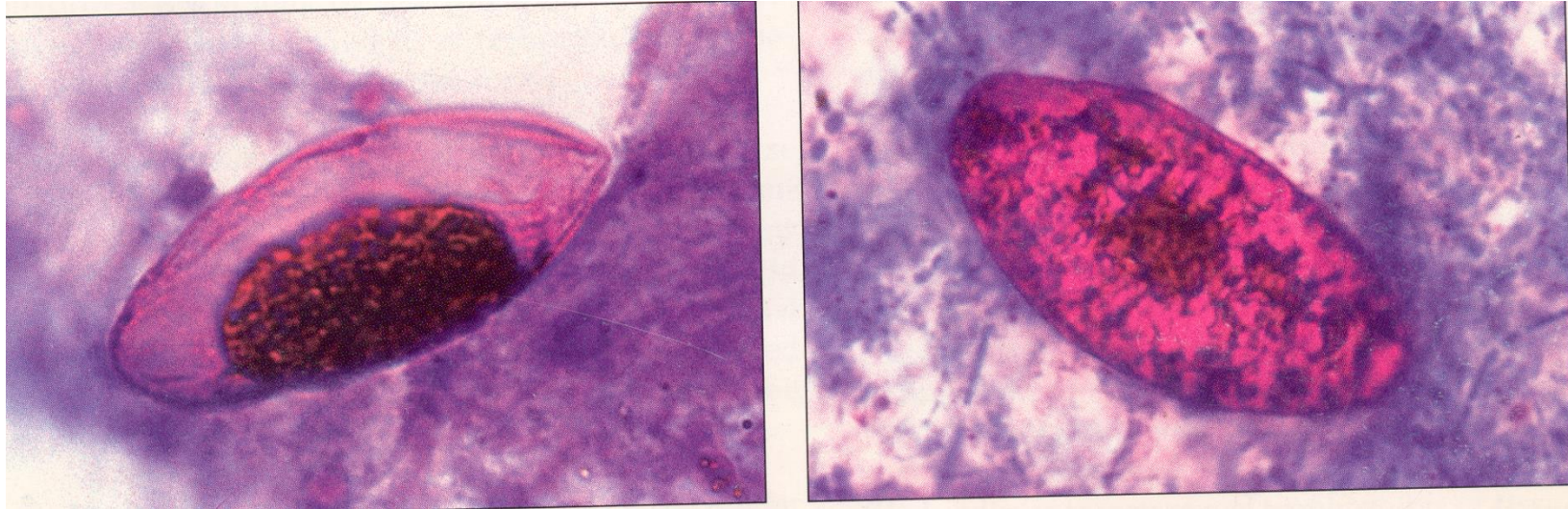
- Infected cell destroyed, parasites released & invade new cells
- Serious infections results in loss of large areas of the gut lining and allow secondary invasion by gut microbes
- At some point, merozoites transform into multinucleate meronts & give rise to microgametes (male) or macrogametes (female)

Life Cycle

- Unless the microgametes & macrogametes occur within the same cell (which is possible), microgametes leave their host cell to locate a macrogamete
- Fusion results in the formation of a zygote that then develops into an oocyst
- Oocysts released into the gut lumen when host cell dies as **sporoblasts** & shed in faeces (not immediately infectious)

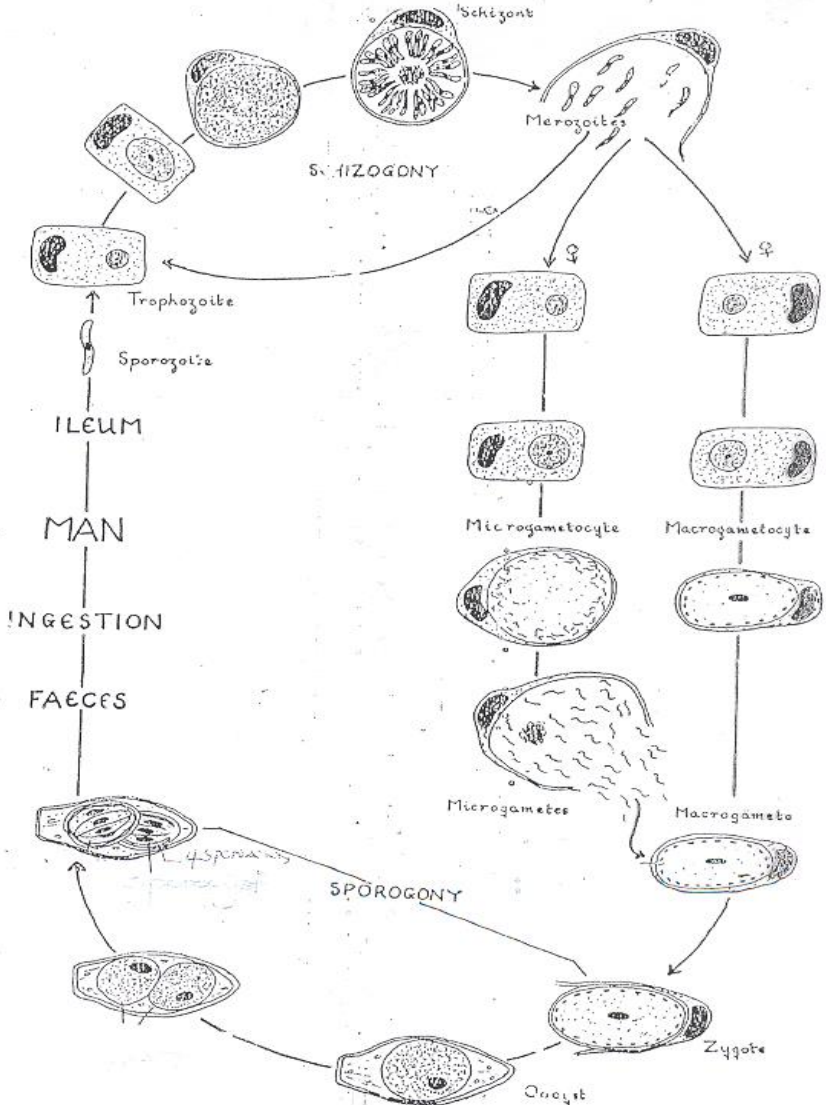
Life Cycle

- Oocysts (**flask-shaped**), one end of oocyst narrower
- Undergo sporulation in the environment to produce **two sporocysts**, each of which contains four sporozoites
- Transmission is therefore passive, by faecal-oral route (ingestion of contaminated food and water)

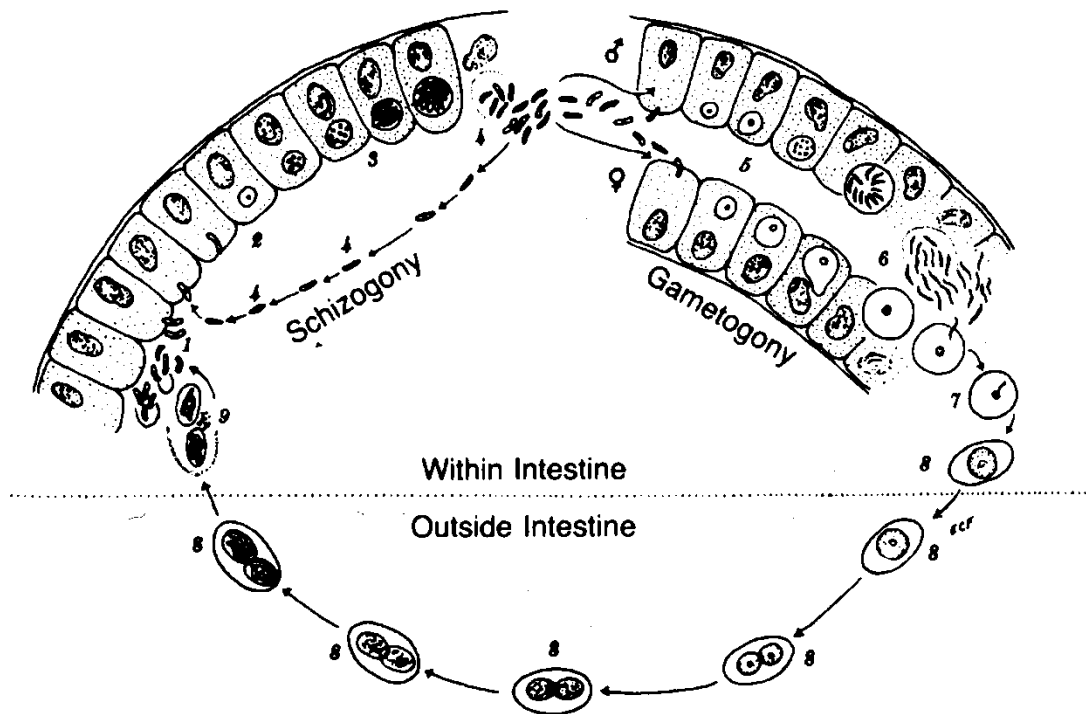


Oocysts of *I. belli* also stain red with acid-fast stains. It is characteristic for the single sporoblast within the oocyst to stain red and for red stain to precipitate along the margin of the oocyst-wall. Left illustrates the typical unsporulated oocyst that is found in fresh feces. Following treatment of patients it is not uncommon for followup fecal specimens to show oocysts that appear to be "empty," ie, lacking the typical sporoblast .

Isospora belli Morphology and Life cycle



Isospora belli life cycle. 1. Sporozoites entering intestinal mucosa. 2, Young trophozoite in mucosal epithelial cell. 3. Development of a schizont. 4, Merozoites shed from disrupted cell. 5, Gametocytes, male (above) and female (below), developed from merozoites. 6, Gametes. 7, Union of gametes to form zygote. 8, maturing stages of sporoblasts and oocysts. 9, sporocysts shed from oocyst.

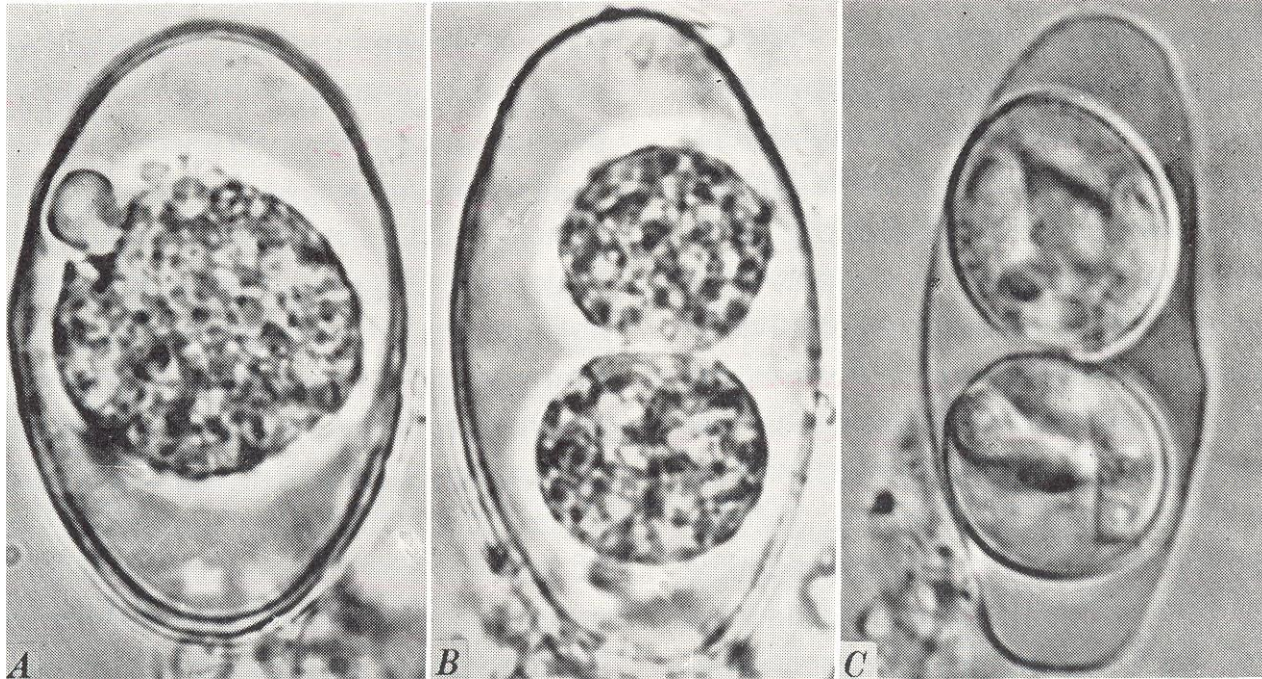


Isospora belli.

A, Oocyst from freshly passed feces.

B, Maturing oocyst with two sporoblasts.

C, Mature oocyst with two sporocysts each containing four sporozoites.



Pathogenesis

1. Asymptomatic carriers
2. Coccidiosis: chronic diarrhoea, mucus secretion
3. Associated with malabsorption syndrome
4. Shortened microvilli, hypertrophied crypts
5. Some ulceration, fever, infiltration with eosinophils, and Charcot-Leyden crystals

Symptoms

1. Weight loss, anorexia, malaise and fatigue, dehydration
2. Loose, fowl smelling stool

Diagnosis

1. Presumptive diagnosis (clinical)
2. Finding oocysts in faeces by direct smear or modified Ziehl-Neelsen stain – bright red

Treatment

- Trimethoprim
- Pyrimethamine and Sulphadiazine
- Co-trimoxazole 960mg 4 times daily orally for 10 days

Prevention and control

:- as in fecal-borne infections