

TUCOM

Year 3

Practice Parasitology

Lab. 6



Cryptosporidium parvum

Learning objectives:-

At the end of this lab the students should be able to:-

1. Describe the morphology forms of thick-walled oocyst of *C.parvum*.
2. Briefly describe the life cycle of *C.parvum* .
3. Identify the laboratory diagnosis of *C.parvum* .

Phylum: Apicomplex

Class: coccidia

Introduction:

- *Cryptosporidium* is a coccidian protozoan parasite(Sporozoa)
- Associated with diarrhoea in 1976 in a 3-year-old girl in EM of the intestinal epithelium
- Increasing population of immunocompromised persons and waterborne outbreaks of cryptosporidiosis,
- Recent occurrence of cryptosporidial acute watery diarrhea in ADIS patients
- Self –limiting diarrhea in immunocompetent person
- Faecal-oral transmission (coccidian type life cycle)
- Two species infecting humans

C.parvum: cattle and human ,other mammals(zoonotic transmission)

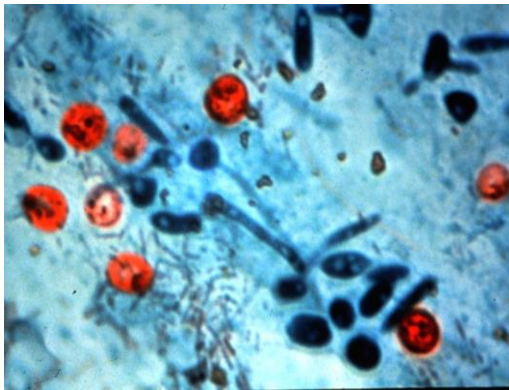
***C.hominis*: only humans(anthroponitic transmission)**

- Besides gastrointestinal infection extra-intestinal infection of respiratory tract have been reported in severely immunocompromised patient also in immunocompetent person
- *Cryptosporidium* can infect several different hosts(domestic calves,birds,and mice) clearly indicating that sporodiosis as zoonosis, and the parasite can survive most environments for long periods .

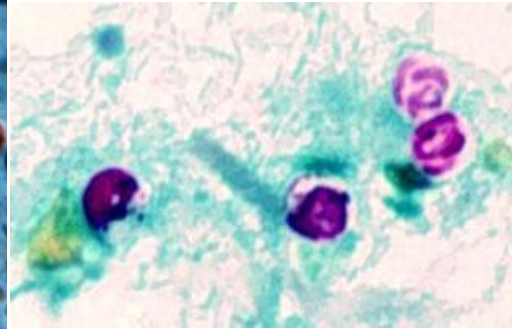
Habitat : The parasite is intra-cellular and found in distal parts of jejunum,ileum the and also the colon.

Morphology :

Oocyst the infective form of the parasite . The oocyst exists in two forms, namely one thick-walled (80%) and other thin(20%).Oocysts are spherical or oval, colourless ,measuring 1.5-5µm in diameter,highly refractile and are shed in th stool. Four elongated sporozoites are present in both thick-walled and thin-walled oocyst.Thick-walled oocyste excerted in the faeces and thin-walled oocyst causes autoinfection.



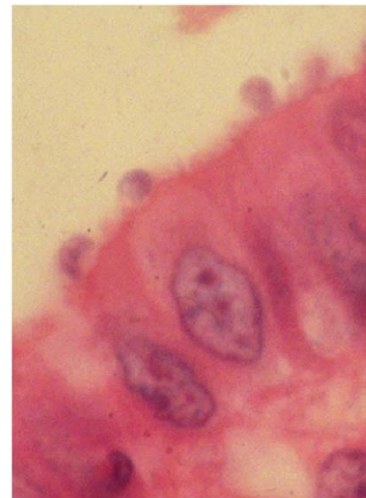
C. parvum - Cysts in stool Acid fast



Oocysts of *Cryptosporidium parvum* stained by the modified acid-fast method. Against a blue-green background, the oocysts stand out in a bright red stain. Sporozoites are visible inside the two oocysts to the right.



A scanning electron micrograph of a broken meront of *Cryptosporidium* showing the merozoites.



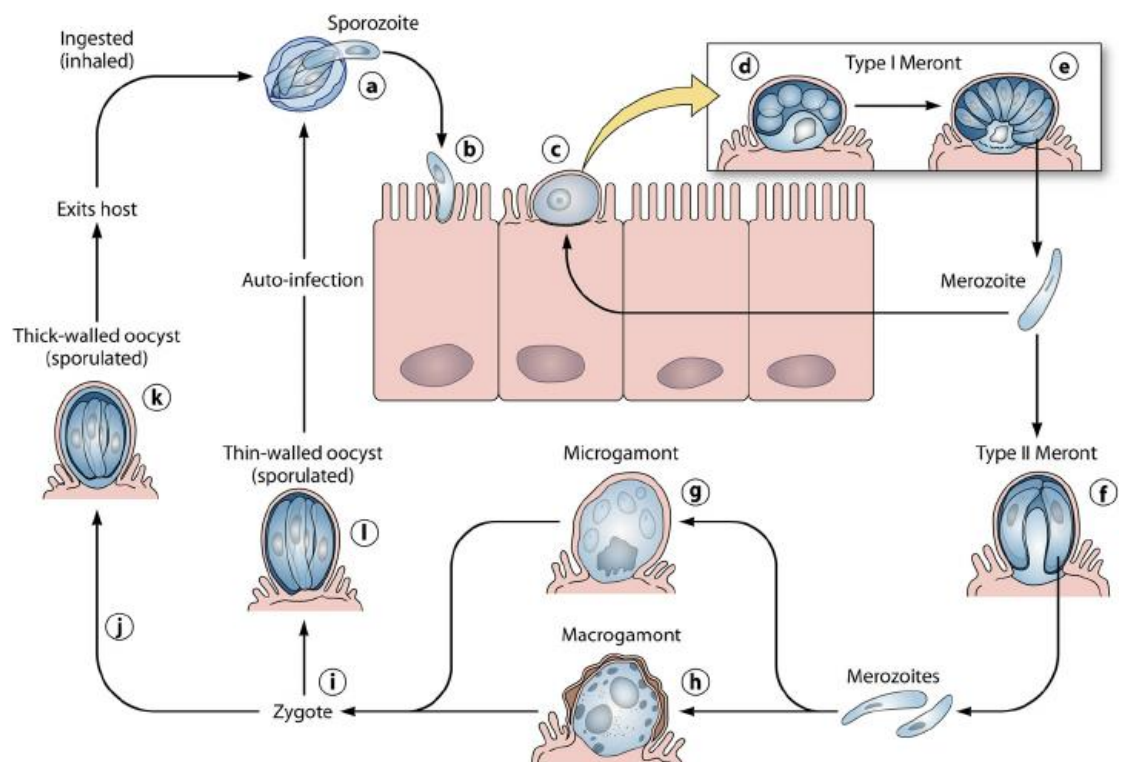
Intestinal biopsy demonstrating *Cryptosporidium parvum* at the surface of the intestinal epithelial cells. Hematoxylin and eosin stain, 100X oil objective

Life cycle:

Complex monoxenous life cycle--completing its entire cycle within a single host with both sexual and asexual cycles, the normal life cycle of the parasite is similar to *Isospora belli* except that the parasite does not have an intracellular stage; all the development takes place on the surface of the mucosal cells.

There are six distinct developmental stages:-

1. Thick-walled oocyst(80% excreted in faeces)
2. Thin-walled oocyst(20% causes auto-infection)
3. Type I meront(With 8 merozoites)
4. Type II meront(With 4 merozoites)
5. Macrogamont(produce only one macrogamete)
6. Microgamont(each macrogamete produce 12-16 microgamete)



Schematic representation of the *Cryptosporidium parvum* life cycle. After excysting from oocysts in the lumen of the intestine (a), sporozoites (b) invade the brush border of epithelial cells and develop into trophozoites (c) within parasitophorous vacuoles confined to the microvillous region of the mucosal epithelium.

Trophozoites undergo asexual division (merogony) (d and e) to form type I merogont with 8 merozoites. After being released from type I meronts, the invasive merozoites enter adjacent host cells to form additional type I meronts or to form type II meronts (with four merozoites) (f).

Type II meronts do not recycle but enter host cells to form the sexual stages, microgamont (one microgamont produce 12-16 microgametes) (g) and macrogamont (one macrogamont produce only one macrogamete) (h).

Most of the zygotes (i) formed after the fertilization of the macrogamete by the microgametes (released from the microgamont) develop into environmentally resistant, thick-walled oocysts (j) that undergo sporogony to form sporulated oocysts (k) containing four sporozoites.

Sporulated oocysts released in feces are the environmentally resistant life cycle forms that transmit the infection from one host to another. A smaller percentage of zygotes (approximately 20%) do not form a thick, two-layered oocyst wall; they have only a unit membrane surrounding the four sporozoites.

These thin-walled oocysts (l) represent autoinfection life cycle forms that can maintain the parasite in the host without repeated oral exposure to the thick-walled oocysts present in the environment.

Detection and Diagnosis

- 1-Staining methods were then developed to detect and identify the oocysts directly from stool samples ,also in sputum.
- 2-The modified acid-fast stain(Zeihl-Neelsen method) is traditionally used to most reliably and specifically detect the presence of cryptosporidial oocysts
- 3-ELISA or IFA, has recently been described in diagnosis of cryptosporidiosis
- 4-PCR (Polymerase Chain Reaction) has been used for detection DNA *C. parvum*
- 5- Intestinal biopsy material demonstrates various stages of *C. parvum*

Prevention and control:

similar to *Isospora belli*

Treatment:

Disease is self limiting but sometimes ,particular in ADIS patient, effective treatment with nitazoxanide,in a dose of 500mg twice daily for 3-5 days.

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