Cryptosporidiosis

Although the first human cases of Cryptosporidium were described in 1976, the contribution of this protozoan parasite to gastrointestinal disease was not fully appreciated until the 1980s when scores of cases were described among patients with acquired immunodeficiency syndrome (AIDS). The disease gained greater notoriety after a massive outbreak of waterborne cryptosporidial infection in Milwaukee, Wisconsin in 1993. Watery diarrhea and malabsorption are the usual sequelae of symptomatic infection. In addition to Cryptosporidium causing chronic diarrhea and extraintestinal disease in immunocompromised individuals, the parasite is an important source of self-limited diarrhea in immunocompetent individuals and a major contributor to persistent diarrhea in children throughout the developing world. Limited therapeutic options for persistent and chronic disease present an additional challenge to clinicians and treatment of any underlying immunodeficiency is paramount.

The genus Cryptosporidium consists of at least 10 species. This group of organisms resides within the subphylum Apicomplexa, along with other protozoan parasites such as Plasmodium species. It is most closely related to coccidian parasites including other intestinal pathogens such as Cyclospora and Isospora species. Cryptosporidium species have been detected in the gastrointestinal tract of a number of mammalian and vertebrate species. Its presence in ruminants has been most widely described. C. parvum, a species commonly found in bovine hosts, was formerly the species most often associated with human disease. However, genotypic and phenotypic differences among isolates eventually led to the recognition of two separate species, C. hominis (formerly “human” genotype or C. parvum genotype 1) and C. parvum (“bovine” genotype or C. parvum genotype 2). Humans are most commonly infected by C. hominis or C. parvum and on occasion by species normally present in other animal hosts. It appears that C. hominis only infects humans.

Mixed infections have been rarely described in immunocompromised patients. As the genetic diversity of various host-adapted species is better appreciated, it is likely that the present nomenclature will evolve and the

relative importance of zoonotic transmission will undergo further re-evaluation.

Lifecycle:-

The pattern of Cryptosporidium life cycle fits well that of other intestinal homogeneous coccidian genera of the suborder Eimeriina: macro- and microgamonts develop independently; a microgamont gives rise to numerous male gametes; and oocysts serving for parasites' spreading in the environment.

Electron microscopic studies made from the 1970s have shown the intracellular, although extracytoplasmic localization of Cryptosporidium species.

These species possess a number of unusual features:

1- an endogenous phase of development in microvilli of epithelial surfaces

2- two morphofunctional types of oocysts

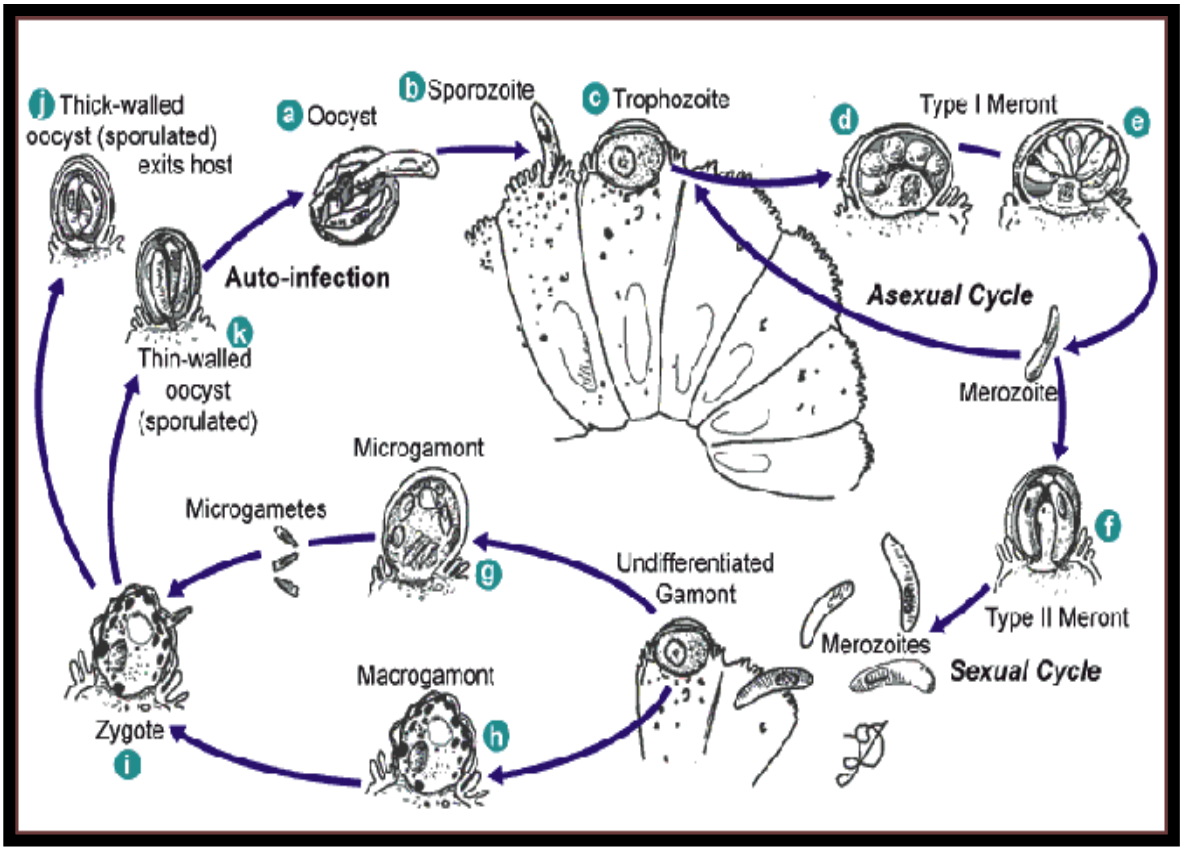
3- the smallest number of sporozoites per oocyst

4- a multi-membraneous "feeder" organelle

The life cycle of Cryptosporidium can be completed entirely within a single host . Subsequent to oocyst ingestion and activation in the upper GI tract, the organisms excyst to release sporozoites. These sporozoites bind intestinal epithelial cells and via induction of actin polymerization, provoke their own engulfment to eventually reside in a parasitophorous vacuole within the microvillus layer. In this sequestered environment, the parasites undergo asexual reproduction (termed merogony) and ultimately produce merozoites that are released intra luminally.

These may either penetrate a new epithelial cell to repeat the cycle (type 1 meront contain 8 daughter merozoites) or undergo further intracellular changes (type 2 meront contain 4 daughter merozoites) to the sexual form of the parasite.

Alternatively the engulfed merozoites may undergo sexual differentiation and ultimately the fertilization of macrogamonts by microgametes will yield new oocysts. These new oocysts may either be shed into the environment or excyst within the same host.



C. parvum induces apoptosis is the process of programmed cell death in epithelial cells. It is assumed that an enterotoxin is produced, but this has not yet been proven. The immune system reduces the formation of Type 1 merozoites as well as the number of thin-walled oocysts. B cells do not help with the initial response or the fight to eliminate the parasite. The parasites may be found throughout the entire digestive tract and even in the mucosa of the respiratory tract, but are usually limited to the duodenum and jejunum.

Pathogenesis and the Host Response:-

The incubation period is usually 7-10 days and is followed by moderately severe diarrhoea without fever and with little abdominal pain but no particular characteristics. Asymptomatic infections may occur. If there is no underlying immunosuppression, spontaneous recovery occurs within a few weeks. It is estimated that 4 to 10% of all commonplace cases of diarrhoea in children in tropical environments can be attributed to Cryptosporidium. In patients who have a deficiency in cellular immunity (such as in HIV infection), the diarrhoea is more pronounced, chronic for several months, and recurrent. This is accompanied by painful abdominal cramps, nausea, dehydration, loss of weight and mild fever.

The 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. Cholangiocytes are also susceptible to infection, and apoptosis of these epithelial cells likely contributes to biliary tract disease. The respiratory tract also appears to be a site of infection in immunocompromised individuals. Epithelial cell death, by both apoptotic and necrotic mechanisms, has been noted in involved regions.

Non bloody diarrhea is the most common clinical presentation of cryptosporidiosis; however clinical findings may vary widely and are dependent on the affected host population being considered. The severity and duration of diarrhea may be quite variable. The incubation period is usually 7 to 10 days, though it can range from several days to weeks.

Epidemiology

Cryptosporidium has a wide geographic distribution, though infection is more prevalent in regions of the world with poor sanitary conditions. Infection is more common during warm rainy months. The reported prevalence of infection varies widely and is influenced by geographic region, age, immune status, local outbreaks and the range of sensitivities and specificities offered by different diagnostic modalities. In general, exposure rates based on seroprevalence studies suggest that in North America at least 30% of adults have been previously exposed to Cryptosporidium species. However, seroprevalence rates are as high as 90% in the developing world.

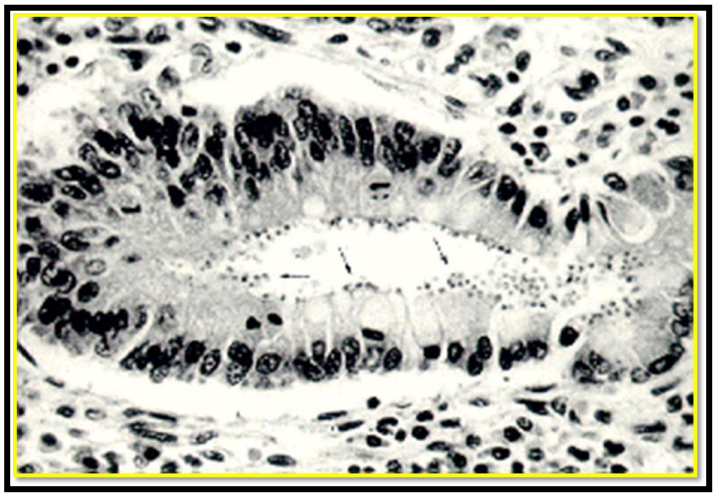
The Immunocompromised Host: AIDS and Other Immunodeficiencies:-

In the patient with HIV, the course of cryptosporidiosis often correlates with the immune status of the individual. Patients with CD4 counts above 200 cells/µL are likely to have a clinical course similar to immunocompetent hosts. Patients with AIDS and progressively declining CD4 counts are more likely to present with foul smelling bulky stools in the context of chronic diarrhea and weight loss. Severely immunocompromised individuals with CD4 counts less than 50 cells/microL develop a more fulminant cholera-like disease with watery and voluminous diarrhea. Biliary and respiratory tract disease is more likely to manifest in severely immunocompromised persons with CD4 counts less than 50 cells/µL. Biliary tract involvement may result in

biliary strictures, papillary stenosis, pancreatitis, acalculous cholecystitis, or sclerosing cholangitis. These may manifest with right upper quadrant pain, nausea, vomiting and low grade fever.

Although oocysts have been detected in respiratory secretions of immunocompromised patients, a causal link between Cryptosporidium and pulmonary disease is usually difficult to establish given the occurrence of coexistent opportunistic pathogens in this population.

More severe or persistent cryptosporidiosis has also been described in other immunocompromised settings, including organ transplantation, immunosuppressive therapy, chemotherapy, primary immunodeficiencies, hematologic malignancies, cytokine deficiencies and a variety of other conditions associated with cell mediated immune dysfunction. CD4 T-cells and the induction of certain cytokines, particularly interferon-gamma, play a critical role in controlling infection.

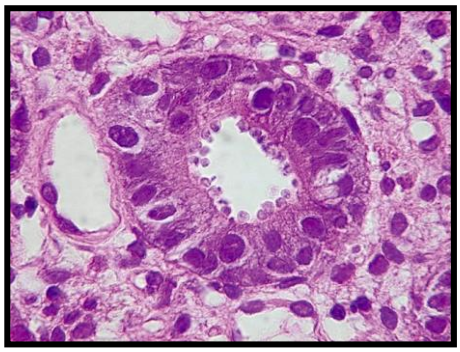


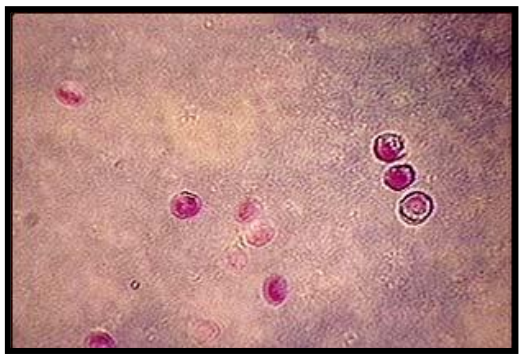
Diagnosis:-

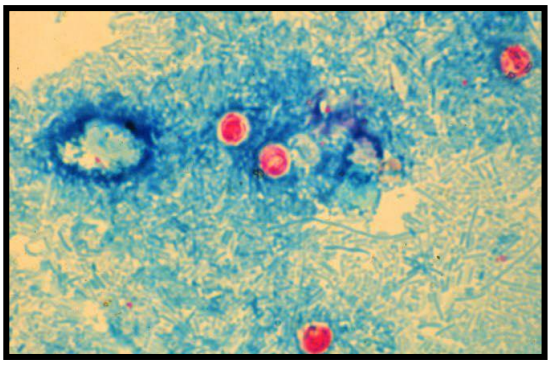
There are many diagnostic tests for Cryptosporidium. They include microscopy, staining, and detection of antibodies. Microscopy can help identify oocysts in fecal matter. To increase the chance of finding the oocysts, the diagnostician should inspect at least 3 stool samples. There are several techniques to concentrate either the stool sample or the oocysts.

The modified formalin-ethyl acetate (FEA) concentration method concentrates the stool. Both the modified zinc sulfate centrifugal flotation technique and the Sheather's sugar flotation procedure can concentrate the oocysts by causing them to float. Another form of microscopy is fluorescent microscopy done by staining with auramine. Other staining techniques include acid-fast staining, which will

stain the oocysts red. One type of acid-fast stain is the modified Ziehl-Neelsen Kinyoun technique. Giemsa staining can also be performed.
Part of the **small intestine** can be stained with hematoxylin and eosin (H & E), which will show oocysts attached to the epithelial cells.

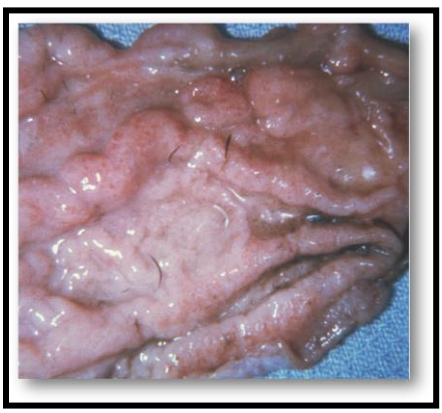






Detecting antigens is yet another way to diagnose the disease. This can be done with direct fluorescent antibody (DFA) techniques. It can also be achieved through indirect immunofluorescence assay. Enzyme-Linked ImmunoSorbent Assay (ELISA) also detects antigens.
Polymerase chain reaction (PCR) is another way to diagnose cryptosporidiosis.

The small dimensions of the parasites and their similarity to yeast cells, were responsible for the fact that infection in humans was only recognized in 1976.
The parasites can easily be recognized on intestinal biopsy material obtained by endoscopy. There is villous atrophy, hyperplasia of crypts and an inflammatory cellular infiltrate in the lamina propria.



Treatment

- Paromomycin
- Azithromycin and letrazuril
- Self limited in immunocomptent persons ,no effective drugs in cases of AIDS.
- Management of fluid and electrolytes loss.

Isosporosis

Isospora belli was first described by Virchow in 1860 but not named until 1923. It is a coccidian parasite of the duodenum and proximal small intestine (jejunum) in humans.It is cosmopolitan, but more frequent in a tropical environment. No reservoir hosts other than man are known. The oocysts are very resistant to environmental conditions and may remain viable for months if kept moist. The sexual and asexual cycles occur in the same host. The parasites are located intracytoplasmic, unlike Cryptosporidium. There is a prepatent period of about 9-10 days. Infection may be latent or lead to diarrhoea for one to two weeks, occasionally with mild fever, headache, malaise and abdominal pain.

The stools tend to be soft, watery or foamy, with an offensive smell, suggesting malabsorption. In immunosuppressed people, the infection can become chronic. In such cases, oocyst shedding can continue for years. In AIDS patients, the parasites can occasionally be found in lymph nodes and walls of large and small intestine, mesenterium and even liver and spleen. Diagnosis is difficult and is based on coprological examination, duodenal tubage and biopsy of the duodeno-jejunal mucosa, in which the parasites are not very numerous. The oocysts are rather large and measure 20-33 μm by 10-19 μm.

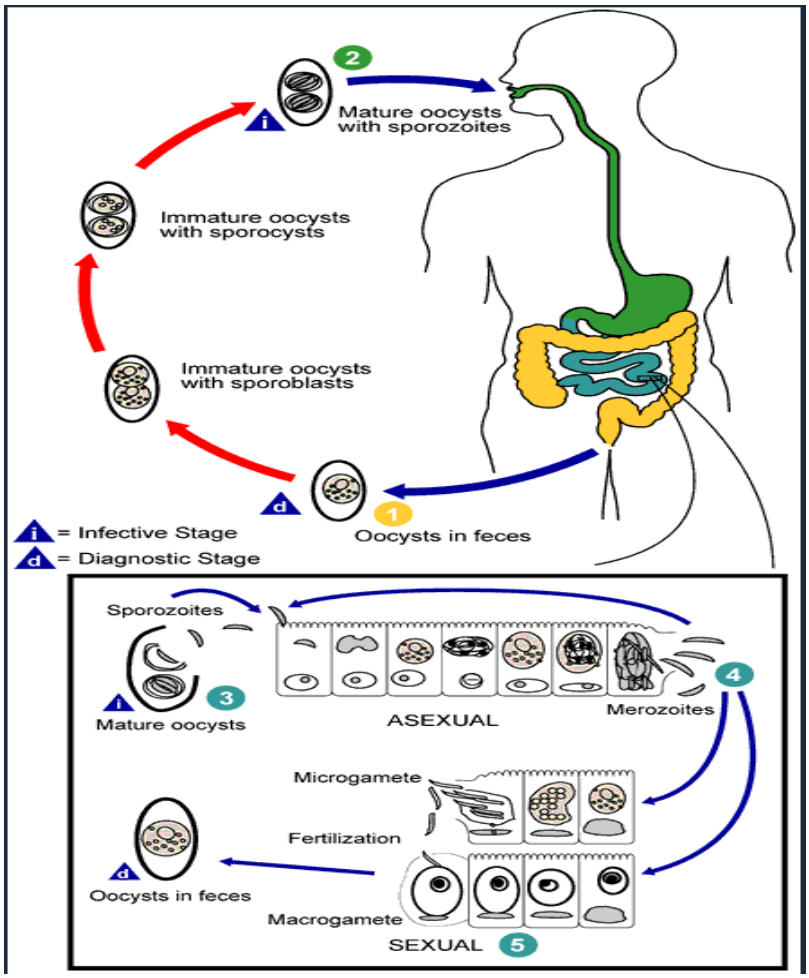
Diagnosis :-

The oocysts are very pale, transparent and are easily overlooked, especially in a concentrated sediment of a polyvinyl alcohol-preserved stool sample. For this reason, it is best to diminish the light intensity of the microscope and additional contrast should be obtained for optimal examination conditions.

Wet preparations are generally preferred. Charcot-Leyden crystals (derived from eosinophils) are occasionally found in isosporiasis cases. The oocysts are acid-fast and can also be detected with auramine-rhodamine staining. Usually the oocyst contains only one immature sporont, but two may be present. Continued development occurs outside the human host with the development of two mature sporocysts, each containing four sporozoites. Normally this takes about 48 hours. The sporulated oocyst is the infective stage which will excyst in the duodenum.

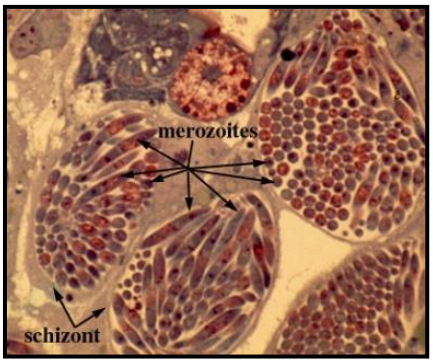
Treatment

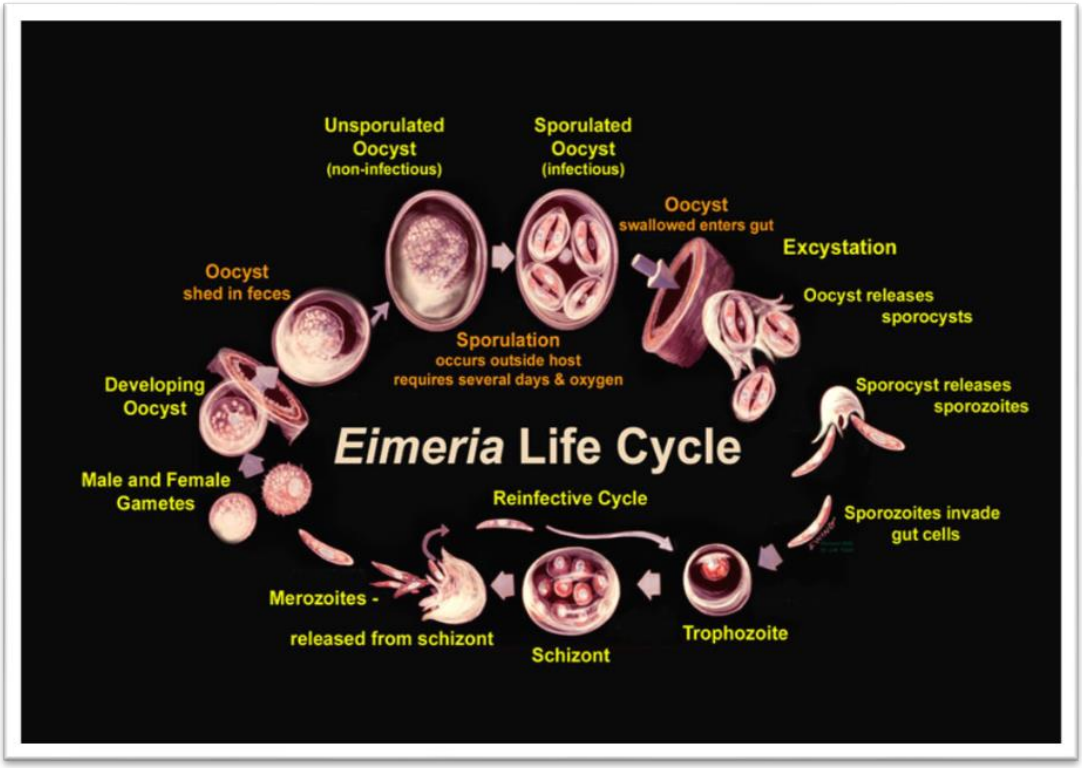
- The condition can be treated with cotrimoxazole
- pyrimethamine (Daraprim)
- the combination ornidazole/albendazole is used.
- Ciprofloxacin is also moderately effective.
- Prevention is based on improved personal hygiene measures and improvement of the sanitary conditions.



Eimeria:

- Do not confuse with Isospora hominis (pathogenic) or Eimeria, a genus not pathogenic for humans. Eimeria sp. form the largest and the most economically important genus of Coccidia and includes species which can infect various mammals and birds. How long the protozoa survive, the period of sporulation under various circumstances, the infectious dose (probably low), the existence of alternative routes of transmission than faeco-oral transmission are all insufficiently clear.



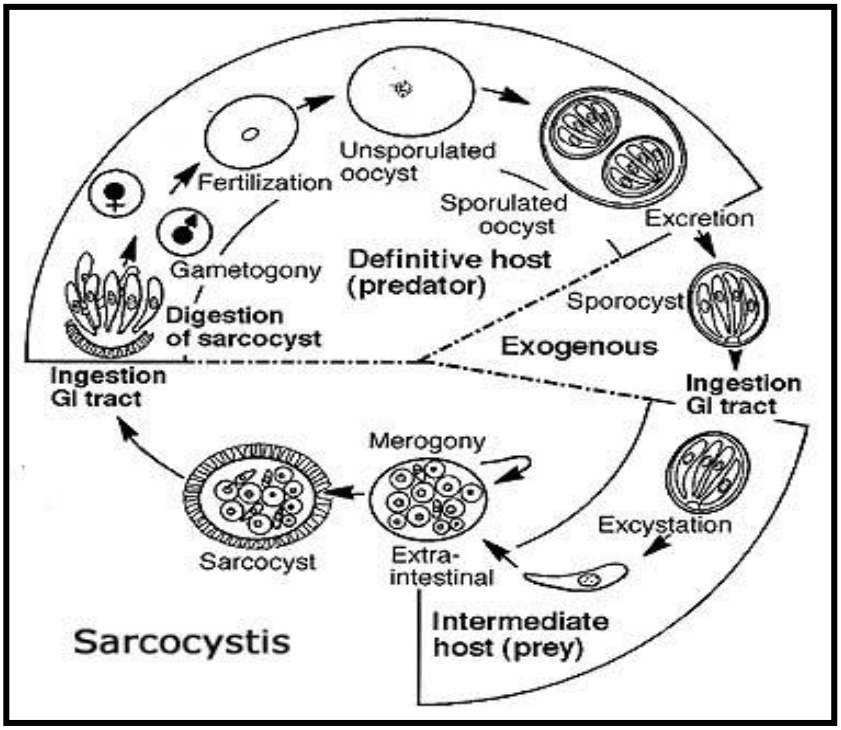


Sarcocystosis:-

Sarcocystis species are parasites of mammals, birds and reptiles. Human sarcocystosis (syn. Sarcosporidiosis) is rarely diagnosed. The parasites almost always have a life cycle involving 2 hosts. Sexual reproduction (gametogony followed by sporogony) takes place in the intestine of a carnivore or omnivore (the predator). Asexual reproduction (schizogony) takes place in the muscles of another host (the prey).

In 1843 Miescher described the parasite for the first time as white threads in the muscles of a mouse. In 1869 Lindemann erroneously described 3 human cases. In 1893, Kartulis described the first authentic human case. In 1972 it was discovered that the intestinal parasite Isospora hominis and the muscle parasite Sarcocystis hominis were 2 stages of the same organism (do not confuse with Isospora belli).

For some species, humans are the definitive host i.e. the host in which sexual reproduction is completed. In this case there is intestinal sarcocystosis. Humans may also act as accidental dead-end intermediate hosts for several other species and in these cases there is muscular sarcocystosis. The natural final and intermediate hosts of many Sarcocystis species, which infect human muscle are still unknown. The Sarcocystis species themselves are also still unknown. Histologically the tissue cysts are often similar to those found in local monkeys. All in all, little is known about these parasites.

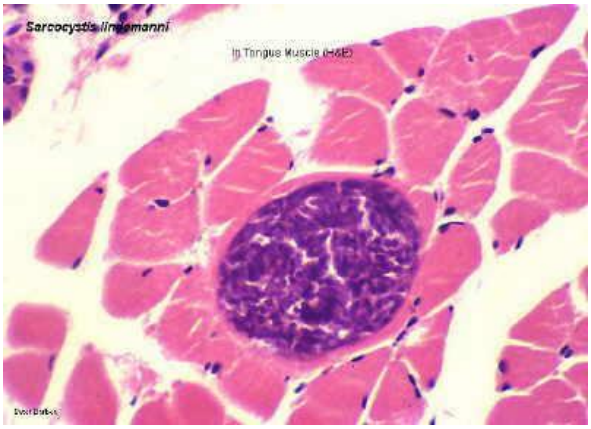


Intestinal sarcocystosis

Sarcocystis bovihominis and Sarcocystis suihominis are parasites of humans. Infection occurs due to eating raw or insufficiently cooked meat from cattle or pigs containing tissue cysts. The sexual cycle takes place within the cytoplasm in the cells of the human intestinal mucosa. The sporocysts which are released with the faeces are infectious for the intermediate host. These infections are cosmopolitan and generally asymptomatic. They can nevertheless trigger enteritis with peripheral hypereosinophilia. The diagnosis is based on faecal examination. Sometimes the parasites will be detected in surgical resected intestinal specimens.

Muscular sarcocystosis

Muscle infection is caused after swallowing sporocysts (faeces of an infected predator). Each sporocyst releases 4 sporozoites. These penetrate the intestinal wall. Reproduction begins in the vascular endothelium. After dissemination of merozoites, there is invasion of skeletal and cardiac muscle tissue and possibly the central nervous system (in animals). The merozoites develop first to metrozoites and then to cystozoites. These tissue cysts remain dormant until the host is eaten by a predator, after which the intestinal cycle begins.



Most human infections are apparently asymptomatic. It is also possible that the diagnosis is systematically missed (data from investigation of routine autopsies). No cases of neurological involvement in human patients are known. Some patients with muscular sarcocystosis develop an eosinophilic myositis. The myositis is characterised by muscle pain, painful mild muscular swelling, mild

fever, general weakness, bronchospasms and eosinophilia. This should be differentiated from trichinosis (Trichinella spiralis). Eosinophilic fasciitis, toxoplasmosis, polymyositis, dermatomyositis and polymyalgia rheumatica may lead to similar clinical pictures. During the short blood phase, the parasites can be mistaken for Plasmodium falciparum gametocytes, or even a rare blood form of Toxoplasma gondii.

Diagnosis is made via muscle biopsy. The intact cysts in the muscle generally do not trigger a local inflammatory reaction. Dead and ruptured cysts, however, may cause inflammation. It is necessary to differentiate from tissue cysts of Toxoplasma gondii (Toxoplasma generally has smaller cysts with a thin non-striated capsule) or Trypanosoma cruzi. Not much is known about treatment.

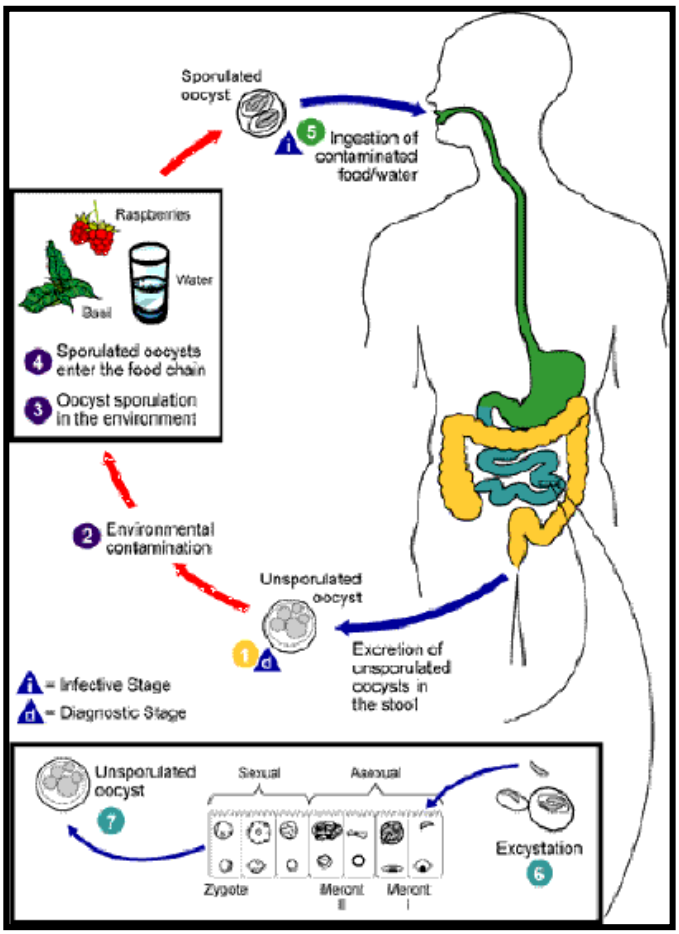
Cyclospora cayetanensis

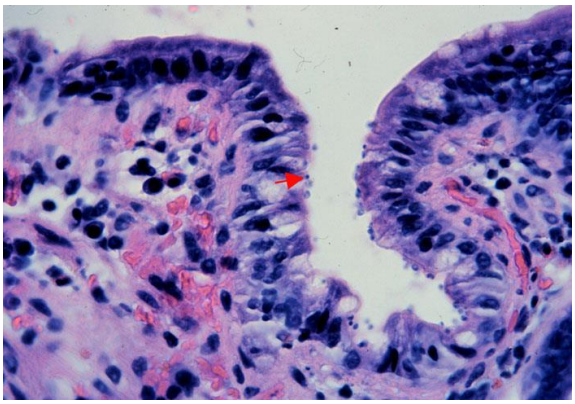
Cyclospora cayetanensis is a protozoon which belongs to to the Coccidia. It is closely related to Eimeria. The name is derived from the morphology (the sporocysts are spherical) and from a Peruvian university (most of the epidemiological and taxonomic work has been carried out at the Universidad Peruana Cayetano Heredia, Lima, Peru). Before 1992 the organism was wrongly regarded as a cyanobacterium. Distribution is probably cosmopolitan, but the species is only common in regions with poor hygiene.

After swallowing mature (i.e. sporulated) oocysts, there is excystation after contact with bile salts. The released sporozoites penetrate the jejunal enterocytes. In 1997 it was demonstrated that during infection of humans two different asexual stages occur as well as sexual stages. The full cycle can be completed in one host. Infected persons eliminate non-sporulated oocysts in their faeces. Until they sporulate, which takes days or weeks, these parasites cannot infect a new host. This delay makes direct human to human transmission improbable. After sporulation the oocyst contains 2 sporocysts each with 2 sporozoites. A reminder: In Isospora one oocyst has 2 sporocysts each with 4 sporozoites. Cryptosporidium oocysts contain no sporocysts, only 4 naked sporozoites.

Oocysts are spherical, measure 8-9 μm in diameter and contain granular material when not yet sporulated. They stain with varying degrees of acid

fastness, but they are recognisable even without staining. They can be differentiated from Cryptosporidium oocysts because the latter are smaller. Isospora cysts are also acid-fast but are much larger (20-33 x 10-19 µm). Under UV light Cyclospora displays autofluorescence. Isospora and Cryptosporidium do not autofluoresce. Fluorescence with auramine shows bright yellow disks in Cryptosporidium, but is weak in Cyclospora. Nevertheless in practice these more expensive techniques do not have to be used since diagnosis can be made using standard light microscopy.



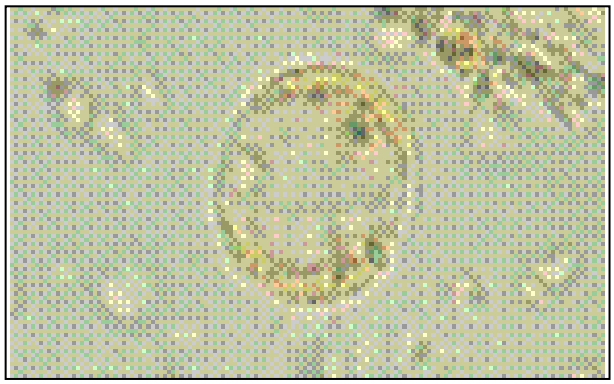


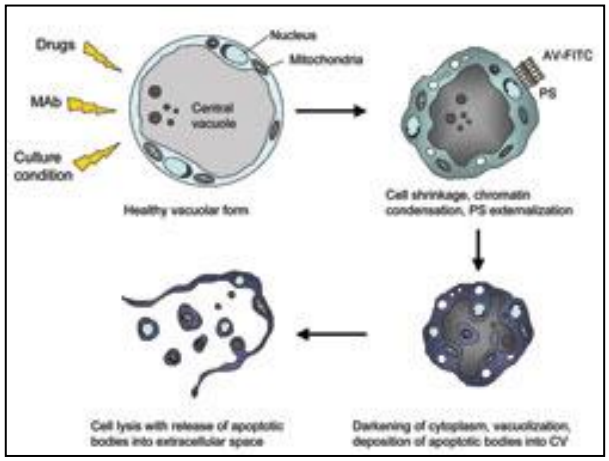
The protozoa are present in the duodenum and jejunum and cause persistent watery diarrhoea, often accompanied by significant abdominal discomfort, nausea, tiredness and anorexia. The symptoms may last several weeks. In particular, non-immune persons such as travellers or small children, will be symptomatic. Cotrimoxazole is used in treatment. This protozoon also causes persistent diarrhoea in HIV-positive persons. If these cannot tolerate cotrimoxazole, the rather less effective ciprofloxacin may be used.

Knowledge about this parasite is insufficient. Transmission via water is possible and food may be contaminated by rinsing vegetables and fruit. Protozoa can be detected in surface water with special techniques. No reservoir is known to date.

Blastocystosis:

- *Blastocystis hominis*, although previously regarded as a yeast, has the morphological and biological characteristics of a protozoon. Very little is known of the basic biology of this organism. Several morphological forms have been recognised: ameboid, vacuolar, avacuolar, multivacuolar, granular, cyst). The life cycle is not well known. Which of the forms is responsable for transmission is not known.





Molecular typing has revealed extensive genetic diversity in morphological identical strains. Until about 1930 it was regarded as a cause of diarrhoea, but thereafter it was generally considered to be an a pathogenic commensal. The parasite colonises chiefly the caecum and to a lesser extent the distal colon. Since 1975, *Blastocystis hominis* has once more been regarded as responsible for long-term but less specific cases of diarrhoea. The

pathogenicity appears to depend on the parasitic load (more than 5 *Blastocystis* per 40x field).

The pathogenicity of *Blastocystis* nevertheless remains controversial. For some clinicians it is only an indication of the presence of one or other parasite or microorganism which is responsible for the symptoms of disease. If considered necessary, imidazoles such as metronidazol or otherwise clioquinol are used for treatment.