Cryptosporidiosis

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Learning Objectives

1. To understand the significance of T-helper cells and innate immunity in protection against *Cryptosporidium* infection.
2. To stress the importance of immunosuppression in causing severe and protracted infection.
3. To make the reader aware about the multiple methods available for diagnosis apart from microscopy.

Introduction

Cryptosporidiosis caused by the coccidian *Cryptosporidium* spp. is one of the most common causes of food and waterborne outbreaks since 2004. The first human case of cryptosporidiosis was reported in 1976. Since then many cases have been reported in both immunocompetent and immunosuppressed hosts. The infection occurs following the ingestion of food and water contaminated with the oocyst of *Cryptosporidium.* The acid-fast sporulated oocysts are the diagnostic form of the parasite. The infection can be prevented by strict hygienic measures.

History

Cryptosporidium was first described in the stomach of mice in 1907. Later E. E. Tyzzer described the motile merozoites of *Cryptosporidium muris* in mouse's gastric epithelium. He named it as genus *Cryptosporidium* (*Crypto* = hidden sporocysts) because unlike other coccidia they did not have sporocyst surrounding the sporozoites. Tyzzer described the morphology and life cycle of another species, *Cryptosporidium parvum*, in 1912. He also described in detail about the developmental stages of the coccidia. The morbidity and mortality due to *Cryptosporidium* diarrhea was first related to the severe diarrheal illness in poultry, caused by *Cryptosporidium meleagridis,* a new species that was first described by Slavin in 1955. It was in the year 1971 that *C. parvum* was reported as a new species causing diarrhea in cattle and lambs. The first case of human cryptosporidiosis was reported in 1976, after which many more cases were reported in both immunocompetent and immunocompromised hosts.

Taxonomy

The genus *Cryptosporidium* belongs to the family Cryptosporidiidae; order, Eimeriidae;

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subclass, Cryptogregaria; class, Gregarinomorphea; phylum, Apicomplexa; and subkingdom, Neozoa, in the kingdom Protozoa.

Cryptosporidium is similar to other coccidia in that they are *monoxenous* (*mon*o, one; *xenous*, host). They are found in the microvillus of epithelial cells in the small intestine but not inside the host cells. They have different stages of development, of which endogenous stage has an attachment organelle, and they are found intracellular but are extra-cytoplasmic.

Genus *Cryptosporidium* has many species that infects a wide range of hosts. These include *C. parvum* (ruminants and humans), *C. hominis* (humans), *C. muris* (rodents and some other mammals), *Cryptosporidium andersoni* (cattle), *C. meleagridis* (birds and humans), *Cryptosporidium baileyi* (chicken and some other birds), *Cryptosporidium canis* (dogs), *Cryptosporidium felis* (cats), *Cryptosporidium galli* (birds), *Cryptosporidium molnari* (fish), *Cryptosporidium wrairi* (guinea pigs), *Cryptosporidium saurophilum* (lizards and snakes), and *Cryptosporidium serpentis* (snakes and lizards). Among all these species, *C. parvum* causes most infections.

Genomics and Proteomics

C. hominis genome is about 9 Mb in size with eight chromosomes. The eight chromosomes range from ∼0.9 to ∼1.4 Mb and exhibit 31.7% GC content compared with 30.3% for *C. parvum.*

C. parvum was previously known as bovine genotype or genotype 2. The previously designated species of *C. parvum* human genotype or genotype 1 or H was currently renamed as *C. hominis*. This was similar in size to *C. parvum* oocyst measuring 4.6–5.4 by 3.8–4.7 μm. There had been a difference in the ribosomal gene expression of *C. hominis* and *C. parvum*, of which the latter expresses two types of rRNA genes (type A and type B), whereas in *C. hominis* more than two transcripts were detected. The genus *Cryptosporidium* has more than 22 species, of which zoonotic *C. parvum* affects both human and animals, while the anthroponotic *C. hominis* also affects both humans and animals. The genotyping is based on SSU rRNA. *C. parvum* and *C. hominis* are further subtyped based on DNA sequence analysis of 60 kDa glycoprotein (gp60 or gp40/15). The subtypes Ia, Ib, Id, Ie, If, and Ig are subtypes of *C. hominis*, and IIa, IIb, IIc, IId, IIe, IIf, IIg, IIh, IIi, IIk, and III are of *C .parvum*. The purpose of knowing the subtypes is to understand about the biological character of the parasite and their difference in clinical presentation.

Protein expression in the soluble fractions of excysted and non-excysted oocysts of *C. parvum* has been reported from one study. A total of 142 proteins were detected in soluble fractions of both excysted and non-excysted oocysts, and ribosomal proteins constituted a significant proportion. Six heat shock proteins and 17 secreted proteins were also expressed. It was noted that many of the detected proteins are involved in infection/pathogenesis, energy pathways, cellular division and replication, and DNA modification.

The Parasite Morphology

Different stages of the coccidian parasites are as follows:

Asexual stage (*sporogony*-inside host cell), sporozoites, Type I meront (8 merozoites), Type II meront (4 merozoites).

Sexual stage *(gametogony*), micro- and macrogametes, zygote, thin- and thick-walled oocysts.

The morphology of the parasite varies according to the stage of development.

The Oocyst

The oocyst which is the infective form is of two types: thin walled and thick walled. They measure about 4–6 μm in size, are round in shape, and are surrounded by a cyst wall. They bear four sporozoites inside. The sporozoite is crescentic in shape with pointed anterior end and blunt posterior end and a nucleus located posteriorly.

Thick-walled oocyst: Thick-walled oocyst is the infective form. It is oval in shape with a smooth surface. It consists of thick and coarse outer wall, fine granular inner wall, and an oocyst membrane in between these two layers. It has a suture point at one end where the sporozoites are released. Electron microscopy shows a double-layered oocyst, outer and inner layers. The oocysts are very resistant to chlorine and other disinfectants.

Thin-walled oocyst: It is similar to the thick-walled oocyst but surrounded by a thin-walled membrane. This stage is responsible primarily for causing autoinfection in humans.

The Sporozoite

The sporozoite measures about 5 × 0.5 μm. They have a rough surface with pointed apical region and rounded posterior end.

The Merozoite

Trophozoite measures about 1–2.5 μm in length, and they have smooth surface. Type I and II meronts vary in size from 1.5 to 3.5 μm, respectively, and the merozoites released by them are of similar in size 0.4 × 1 μm. Type I merozoites are rod shaped with pointed apical region with rough surface, and Type II merozoites have round, rough surface. Microgametes from Type II merozoites measure around 0.1 μm with spherical, rough surface, while macrogametes measure around 4 × 5 μm with oval, rough surface.

Cultivation of Parasites

There has been advancement in in vitro cultivation of *Cryptosporidium* in cell lines. COLO-680 N cell lines infected with two different species (*C .parvum* and *C. hominis*) tend to produce a greater number of infective oocysts, as identified by various microscopic and molecular methods. In vitro culture of *Cryptosporidium* also paves the way for further studies and the development of drugs.

Laboratory Animals

Many animals such as turkeys, chickens, mice, rabbits, rats, guinea pigs, cats, and dogs were used for animal experimental studies. But none of them exhibited symptoms similar to that of cryptosporidiosis.

Life Cycle of *Cryptosporidium* spp.

Cryptosporidium spp. complete their sexual and asexual life cycles in a single host (man or other animals) (Fig. 1).

Hosts

The genus *Cryptosporidium* has more than 30 species, of which around 20 have been identified from humans. *C. parvum* and *C. hominis* cause most human infection. *C. meleagridis*, although an avian coccidian, is considered as the third most important species causing human infections.

Infective Stage

Thick-walled sporulated oocyst causes transmission of infection from person to person, while thin-walled oocyst causes autoinfection in the same infected host.

Transmission of Infection

As few as ten infective *Cryptosporidium* oocysts can cause infection. Humans acquire infection by consumption of food and water contaminated with thick-walled oocyst. The human and animal excreta used as manure for crops contaminate the surface water, ground water, and drinking water sources. Thin-walled oocyst causes autoinfection in the same individual. The thin-walled auto-infective stage contributes to an overwhelming life-threatening infection in immunocompromised hosts.

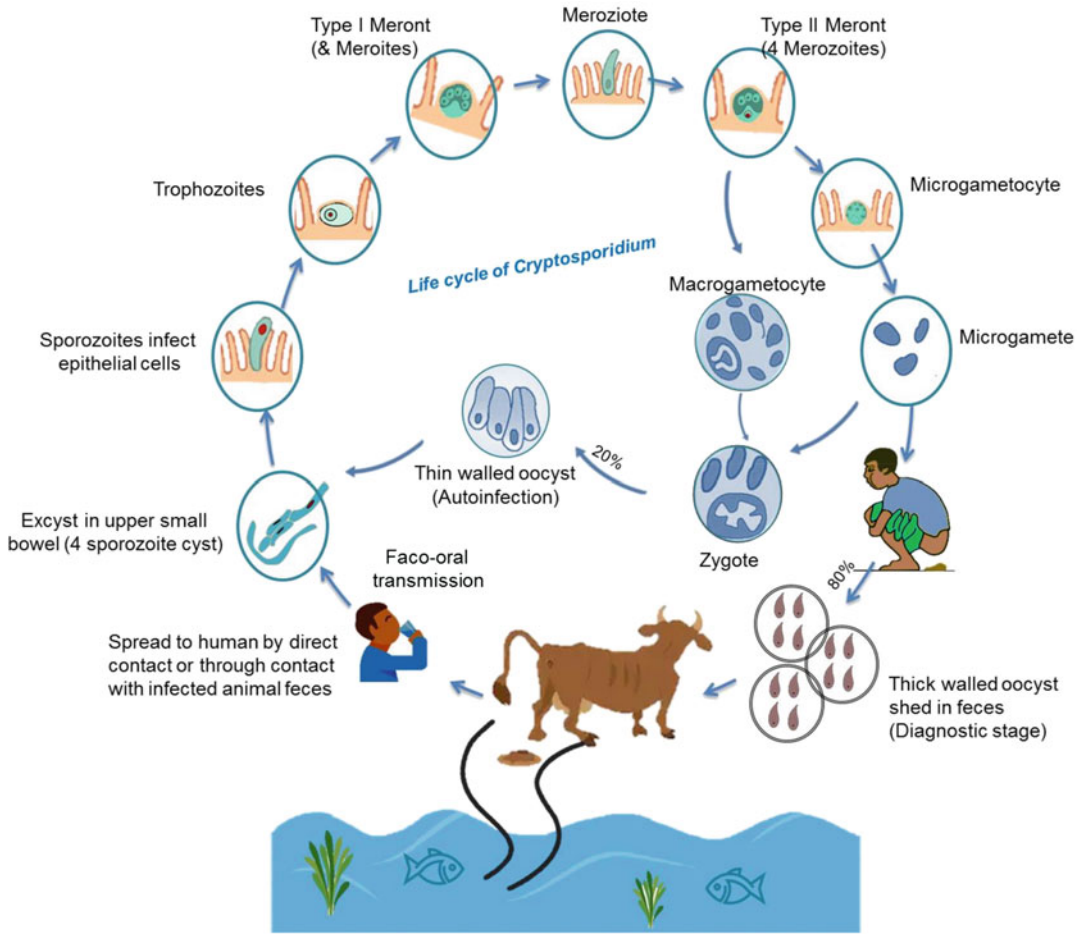

Fig. 1 Life cycle of *Cryptosporidium*

Cryptosporidium completes its life cycle in two different stages: asexual stage (*schizogony*) and sexual stage (*gametogony*) within a single host. Both the stages are intracellular and are surrounded by a host cell membrane which is extra-cytoplasmic.

Asexual Stage (Sporogony)

On ingestion of thick-walled oocysts, the sporozoites are released from the oocyst in the small intestine. Within the enterocytes, the sporozoites develop into intracellular trophozoites, which are the transition stage of the parasite. They multiply asexually by nuclear division to give rise to two types of meronts: Type I and Type II. These meronts in turn produce eight and four merozoites, respectively.

Sexual Stage (Gametogony)

Type II merozoites invade new host cell and undergo sexual reproduction to differentiate into male and female gametocytes. They further divide into macro- and microgametes, respectively, which undergo fertilization to form oocysts or *zygot*e. They form four sporozoites inside the sporulating oocyst. The oocyst is of two types: thin- and thick-walled oocysts. The thin-walled oocyst releases the sporozoites within the lumen of the intestine and causes autoinfection, thus repeating the cycle of sporogony and gametogony. The thick-walled oocysts are excreted in feces and can cause infection in humans, and thus the cycle is repeated.

The life cycle of *Cryptosporidium,* among the coccidian parasites, is different in that they do not

invade deep inside the host cells but are intracellular although extra-cytoplasmic inside the cell. The coccidia are found within the parasitophorous vacuole of the host cell, and the vacuole which contains the organism is present in the microvillus region of the cell surface. The suture wall in the oocyst ruptures, and sporozoites are released inside the small intestine. The sporozoites are released along with the stool in contrast to other similar coccidian parasites, *Cyclospora* and *Cystoisospora.* These parasites undergo excystation only in soil where the temperature is below 37 °C and in the presence of oxygen. Nearly 20% of the oocysts formed are thin walled. After *excystation*, they penetrate the brush border epithelium of small intestine and lie inside the parasitophorous vacuole of microvillus region. They are capable of causing severe and chronic infection in immunocompromised patients even though they are not exposed to environmental oocyst. Around 80% zygote develop into thick-walled oocysts which are resistant to chlorination, and 20% develop into thin-walled oocysts that cause autoinfection.

Pathogenesis and Pathology

Cryptosporidium produces several virulence factors that play important roles in parasite life cycle.

The oocysts in the intestine undergo *excystation* during reducing conditions on exposure to pancreatic enzymes and bile salts. The sporozoites attach to epithelial cells by means of parasite protein, CP47, a 47 kDa *C. parvum* protein. Both sporozoites and merozoites have apical organelles like rhoptries, microneme, dense granules, microtubules, apical rings, and pellicle. Inside the parasitophorous vacuole, there is a change in the apical organelle resulting in attachment to host cells. They cause villous atrophy, mucosal erosion, and depression of the surface mucosa, followed by infiltration of lymphocytes, neutrophils, and plasma cells. The villous atrophy results in D-xylose malabsorption.

Infection of intestinal epithelial cells leads to the activation of nuclear factor kappa B(NF-κB). This activates target genes like antiapoptotic genes such as osteoprotegerin. They allow the parasite to release merozoites before cell death and also activate pro-inflammatory molecules like cytokines and chemokines. The oocysts after causing the infection alter the normal functioning of the intestinal barrier. This in turn causes an increase in the intestinal permeability, absorption, and secretion of fluid and electrolytes resulting in chronic, profuse watery diarrhea especially in immunocompromised hosts. The activation of kinase pathway by the host cells helps in the release of pro-inflammatory cytokines like TNF-α and IL-8. This in turn attracts the phagocytes and leukocytes which can liberate the soluble factors. This results in intestinal secretion of chloride and water and decreases sodium absorption along with glucose transport.

Immunology

Both innate and adaptive immunity play a role in controlling the infection. CD4+ T cells play a major role in AIDS patients.

CD4 cells play an important role in acquired immunity in cryptosporidiosis. The severity and chronicity of *Cryptosporidium* infection depend on the immune status of the individual. Cryptosporidiosis is self-limiting in immunocompetent hosts and in patients with CD4 count >150/μl, chronic in <100/μl, but is life threatening and fulminant in immunocompromised hosts, especially with CD4 count <50/μl. In patients with HIV, the severity of *Cryptosporidium* infection depends on the CD4 cell count.

The role of antibody-mediated immunity in cryptosporidiosis is associated with an increase in IgG, IgM, and IgA to Cp23 in infected individuals compared to healthy, and it gives them longer immunity. IgA production in mothers was found to reduce the rate of infection in children. Monocytes and macrophages which secrete IL-15 and IL-18 were also found to control the infection. IL-18 stimulates IFN-γ production, NK cell activation, and production of

defensins which help in controlling the infection. Interferon γ is also responsible for acquired immunity.

Innate immune response plays an important role in the initial phase of infection. In innate immunity, mannose-binding lectin (MBL) helps in stimulating the immune response. Those children and HIV-infected individual who have MBL deficiency are more susceptible to *Cryptosporidium* infection. Polymorphism in MBL gene results in recurrent infection. The presence of Toll-like receptors on the surface of host cell helps in immune response to the organism. There is an increase in the production of antimicrobial peptides like LL-37 and human β-defensin 2.

Infection in Humans

Incubation period is 1 week. Most of *Cryptosporidium* infections are asymptomatic.

Cryptosporidium infection in immunocompetent individuals with an intact immune system is self-limiting. They usually present with low-grade fever, abdominal cramps, nausea, and anorexia. They have 5–10 times watery, frothy diarrhea with mucous flecks. The symptoms usually subside in 2–3 weeks (Table 1).

Cryptosporidium infection is found to be more severe and persistent in immunocompromised patients with HIV (AIDS); those on chemotherapy and radiotherapy, solid organ, and bone marrow transplant recipients; and patients on immunosuppressive drugs, primary immunodeficiency, etc. The symptoms may last for 7 days to 1 month depending on the severity of illness. It results in significant fluid loss, even up to 25 L. Not only the symptoms involve the gastrointestinal tract, but extra-intestinal sites like the respiratory tract, pulmonary tract, liver, and pancreas may also be involved. These patients do not recover from the illness over a period of time resulting in worsening of their symptoms leading to many complications and even death. The only treatment option for these individuals is to reverse their immunocompromised state rather than with any specific drugs. Survival of patients with CD4 count <200/μl is less compared to those with CD4 count >200/μl. Table 2 compares *Cryptosporidium* infection in immunocompetent and immunocompromised hosts.

Infection in Animals

Cryptosporidium infection is prevalent in a wide range of animals including chicken, mice, turkeys, cattle, dogs, pigs, and sheep. Bovine cryptosporidiosis is distributed throughout the world. Gastrointestinal symptoms are more prominent in cattle. Diarrhea is profuse, watery, and yellow. Fomites such as clothes and footwear used in livestock farm which have been exposed to feces of infected animals can transmit the infection.

Epidemiology and Public Health

Cryptosporidiosis is endemic in the developing countries than in the developed countries. The

Table 1 Clinical presentation of *Cryptosporidium* in immunocompromised and immunocompetent hosts

Characters	Immunocompetent host	Immunocompromised host
Host	Children	Adult
Mode of transmission	Person-person, animal to humans	Person-person
Clinical presentation	Mild to moderate diarrhea, 2–10 times per day	Severe diarrhea, as many as 70 times with fluid loss of up to 12 L/day
Duration of illness	Short, <20 days	Long, months to years
Weight loss	10% of body weight	50% of body weight
Prognosis	Recovery is complete and spontaneous	Usually fatal

Table 2 Distribution of *Cryptosporidium* infection in the global population

Cryptosporidium spp.	Host	Location	Distribution
Cryptosporidium andersoni	Bovines	Abomasum	Malawi
Cryptosporidium felis	Felids, human	Small intestine	India, Kenya
Cryptosporidium hominis	Human	Small intestine	India, Kenya, Malawi, Malawi, South Africa
Cryptosporidium meleagridis	Birds, human	Intestine	India, Kenya, Malawi
Cryptosporidium muris	Rodents, human	Stomach	India, Kenya
Cryptosporidium parvum	Ruminants, human	Intestine	India, Kenya, Malawi, Malawi, South Africa

meta analysis study reported global pooled prevalence of *Cryptosporidium* to be 7.6%. The highest prevalence of infection was reported in Mexico (69.6%), Nigeria (34%), Bangladesh (42.5%), and the Republic of Korea (8.3%) (Table 1). In India, the prevalence of cryptosporidiosis is found to vary from 4% to 13%. The prevalence is higher in semi-urban and rural areas due to poor sanitation. Small, environmentally resistant oocysts, a large number of livestock animals and human reservoirs, low infective dose of <10–100 oocysts, increased multiplication capacity $>10^{10}$, and resistance to available drugs and disinfectants contribute to higher prevalence of cryptosporidiosis in the community. A large waterborne outbreak of diarrhea affecting nearly 4,03,000 people was reported in Milwaukee, Wisconsin, in 1993.

Individuals with HIV, who are immunocompromised due to malignancy, or those on immunosuppressive drugs are more susceptible to infection by *Cryptosporidium* species. Cryptosporidiosis is considered as an AIDS-defining illness (clinical category C) and a category B pathogen by CDC and National Institute of Health. Improper hand hygiene after touching the farm animals is considered an important cause of zoonotic transmission from animals to humans. Nosocomial infection and mechanical transmission through soil and insects are the other modes of transmission.

Laboratory Diagnosis

Microscopy

Stool sample is collected in a wide mouth leak-proof universal container. Three stool samples are collected over a period of 10–15 days since the oocyst shedding in feces is intermittent. Five to six smears per sample are examined to detect *Cryptosporidium* oocysts in infected hosts. Duodenal aspirate, bile, sputum, bronchoalveolar lavage, and biopsy tissues are the other samples examined for the oocysts.

Floatation methods including Sheather's sucrose floatation, zinc sulfate and saturated salt solution, and the sedimentation techniques including formalin-ether and formalin-ethyl acetate methods are various concentration methods used to increase oocyst yield in the stool. Of all these methods, Sheather's sucrose floatation is the most recommended method because it increases higher yield of *Cryptosporidium* oocysts in stool specimens compared to other techniques.

Cryptosporidium oocysts appear as round, double-walled, and refractile bodies, in the saline, iodine, or lactophenol cotton blue wet mount preparations of the stool. Modified acid-fast stain, safranin-methylene blue, negative staining, dimethyl sulfoxide-modified acid-fast staining, immunofluorescence stain, hematoxylin and eosin, and Giemsa and Jenner staining are various permanent staining methods used to detect and identify the coccidian in stool specimens.

The modified acid-fast stain using 1% concentrated sulfuric acid as a decolorizer is the most frequently used method. In the stained smear, the acid-fast oocysts appears as a round, pink-colored structure with four sporozoites and of 4–6 μm in size (Fig. 2). Nearly 50,000–500,000 oocysts/gm of the stool need to be present in order to be visible in the acid-fast stain of the stool smear. The modified acid-fast stain is 83.7% sensitive and 98.9% specific. Table 3 summarizes the differentiating features of *Cryptosporidium*, *Cyclospora*, and *Cystoisospora* in stool specimens. Direct

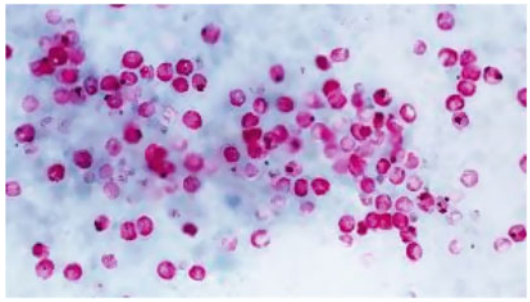

Fig. 2 Acid-fast stain showing oocyst of *Cryptosporidium*

immunofluorescent (IF) stain using monoclonal antibodies specific to cryptosporidial antigen is a highly sensitive method for the diagnosis of cryptosporidiosis (Table 4). Direct IF is considered the gold standard for *Cryptosporidium* in stool microscopy.

Serodiagnosis

Coproantigen Detection

Many serological tests using either native antigen or recombinant antigen of *Cryptosporidium* are available for the detection of serum antibodies in cryptosporidiosis. The antibodies are increased during 6–8 weeks of infection. Antibody detection is mainly useful for sero-epidemiological studies.

Initially, the ELISA was done using crude *C. parvum* extracts; hence, it was less specific than immunoelectrotransfer blot, but now the availability of recombinant *Cryptosporidium* antigens has increased its specificity. ELISA has shown high sensitivity and specificity in studies with various recombinant proteins. ELISA using

Table 3 Differential features of intestinal coccidian parasites

Features	*Cryptosporidium*	*Cyclospora*	*Cystoisospora*
Oocyst size	4-6 μm	8–10 μm	23–36 μm
Shape	Round	Round	Oval
Number of sporozoites	Four sporozoites	Two sporoblast with two sporozoites in each	Two sporoblast with four sporozoites in each
Acid fastness	All oocyst appears as pink acid-fast structures	Variable—some are pink, few oocysts are colorless	All oocyst appears as pink acid-fast structures
Sporulation of oocyst	Occurs inside the host	In soil (environment)	In soil (environment)
Autofluorescence	No	Yes	Yes
Infective form	Sporulated oocyst	Sporulated oocyst	Sporulated oocyst
Diagnostic form	Sporulated oocyst	Unsporulated oocyst	Unsporulated oocyst
Treatment	Nitazoxanide	Cotrimoxazole	Cotrimoxazole

Table 4 Diagnosis of cryptosporidiosis

Diagnostic approaches	Methods	Target	Remarks
Microscopy	Wet mount	Stool saline and iodine wet mount	Similar in size to yeast. Often misinterpreted. Need expertise
	Staining methods	Acid-fast staining, immunofluorescence stain, hematoxylin and eosin, Giemsa and Jenner staining	High parasite load is required to identify Sensitivity – IF>fluorescent stains>AF stain. Direct IF currently a gold standard for stool examination
Serodiagnosis	Immunochromatographic test, ELISA	Oocyst cell wall antigen	Good sensitivity with ELISA
Molecular techniques	Polymerase chain reaction-restriction fragment length polymorphism, multiplex real-time polymerase chain reaction, loop-mediated isothermal amplification	SSU rRNA, gp60, hsp70, 18S rRNA, COWP, and TRAP (C1 and C2	High sensitivity and specificity. Require skilled personnel

a recombinant 23 kDa antigen (Cp23, recombinant form of *C. parvum* 27-kDa antigen) has shown results to the ELISA using native 27 KDa antigen. Serum antibody to Cp23 correlates with past infection while that to Cp17 suggests recent infection. Cp23 has been used to determine longitudinal infection trend and age-specific seroprevalence in cryptosporidiosis. Recombinant rCP41 (cloned and expressed in *Escherichia coli*) has also been used in the ELISA for the seroprevalence studies in cryptosporidiosis.

Rapid immunochromatographic methods are now increasingly used to detect the oocyst cell wall coproantigen in stool specimens for the diagnosis of cryptosporidiosis. The coproantigen is detected by using *Cryptosporidium/Giardia* or triage panel containing *Cryptosporidium/Giardia/Entamoeba*-specific antigens. It is a popular procedure practiced in various laboratories due to its higher specificity (98–100%) and rapid results. ELISA for coproantigen detection has variable detection limits of 3×10^5 to 10^6, which is similar to microscopy. The ELISA, although has the low sensitivity, has the advantage of high specificity of 98%–100%, and large number of samples can be processed in a short time. These tests are suitable to be performed even on fresh, frozen, or formalin-preserved stool samples

Molecular Diagnosis

Molecular methods show high sensitivity by detecting 1 to 10^6 oocysts in stool and other specimens. PCR-restriction fragment length polymorphism (PCR-RFLP), multiplex allele-specific-PCR (MAS-PCR), and quantitative real-time PCR are different nucleic acid-based molecular methods in cryptosporidiosis. 18S rRNA, Hsp 70, TRAP C1, COWP, and DHFR genes are the target genes for *Cryptosporidium* species identification. Further subtype determination can also be done using subtyping tools such as glycoprotein (GP) 60 gene, minisatellite, and microsatellite markers and also by the analysis of extra-chromosomal double-stranded RNA elements.

Nested assay detects most of the common pathogenic *Cryptosporidium* species with the small-subunit rRNA-based PCR-RFLP using external primers of 1325 bp and internal of about 826 bp. Nested assay is the most popular method that has been validated in numerous laboratories globally. The MAS-PCR, which is based on dihydrofolate reductase gene sequence, can differentiate between *C. hominis* (357 bp) and *C. parvum* (190 bp) in a single step and is also useful for detecting small number of oocysts (<100) in the sample. Multiplex assays such as Luminex xTAG gastrointestinal pathogen panel, BioFire FilmArray GI panel, Nano CHIP GI panel, and BD Max parasitic panel are available currently as highly sensitive and specific assays in the detection and identification of *Cryptosporidium* species.

Treatment

Cryptosporidiosis in immunocompetent hosts is a self-limiting disease. The treatment, therefore, is based on fluid replacement with oral rehydration therapy (ORS) or parenteral therapy for seriously ill patients. Chemotherapy by nitazoxanide, paromomycin, and combination of paromomycin (1 g twice daily) and azithromycin followed by monotherapy with paromomycin has been evaluated in the treatment of the condition but with varying results.

Decreasing the dose of immune suppression therapy or strengthening the cellular immune response especially in HIV patients by highly active antiretroviral therapy (HAART therapy) forms the mainstay of treatment of cryptosporidiosis in immunocompromised patients. This helps in increasing their CD4 count. Rifaximin and rifabutin have been tried, but still more studies are required to prove its effectiveness.

Prevention and Control

Prevention is by maintaining proper personal hygiene. Preventive measures include washing hands properly before and after food, after using toilets, and after touching farm animals; avoiding swimming in polluted water, public water park, or

river; washing fruits and vegetables before cooking; avoiding eating uncooked food, and using clean water for washing vegetables. Water purification is important by flocculation and filtration method since chlorination is not effective. Zoonotic transmission can be prevented by wearing gloves and washing hands after handling material contaminated with animal feces and avoiding contact with domestic pets and farm animals especially cattle if they have diarrhea. No vaccine is available.

Case Study

A 42-year-old male who was a post renal transplant recipient on immunosuppressants 1 year back came with a history of chronic refractory diarrhea for past 3 months. He was investigated for complete hemogram; stool examination for ova, cyst, trophozoites; and stool culture before on OPD basis. All investigations were negative. He was treated with antibiotics and anti-parasitic drugs. The patient did not respond to any treatment. The stool was again sent to look for parasites. The modified acid-fast stain showed many round pink irregularly stained structures of approximately 4 μm in size.

1. What is the parasite? What are the other acid-fast parasites seen in stool?
2. What is the commonest artifact in stool with which this parasite can be confused, and how to resolve the issue?
3. What helminthic parasite is also commonly seen in the patients described above?

Research Questions

1. What is the role of humoral immune response in preventing the infection process in cryptosporidiosis?
2. How to assess the existing anti-*Cryptosporidium* drugs to control infections since the existing drugs are not very effective?

Further Readings

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