CRYPTOSPORIDIOSIS
IDENTIFICATION AND TREATMENT AS A REEMERGING DISEASE

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Introduction:
Cryptosporidiosis is a diarrheal disease caused by the parasite Cryptosporidium, which infest the intestine. Only two species infect mammals, C.muris, which primarily infects mice and cattle, and C.parvum, which infects numerous mammals, including human and cattle. C.parvum cause a self limiting infection of the small intestine in immunocompetent human or animal, but it also can be persistent and life threatening in immunocompromised individual, particularly those with AIDS. Chronic cryptosporidiosis became recognized with the emergence of the human immunodeficiency virus (HIV) and AIDS. Viriyavejakul, et al. were found the mayor histopathological change from seventeen HIV-infected by necropsy were cytomegalovirus infection, cryptococcosis, penicillinosis, bacterial pneumonia, cryptosporidiosis and other infections.

Intracellular protozoa:
Over 23 species were originally named in family Cryptosporidiidae based on the host they infected but these different species were subsequently show not to be host specific and are no longer distinguished. Although C.parvum is the most prevalent species causing disease in human infections by C.felis,C.meleagridis, C.canis, and C.muris have also been reported.

Figure 1 shown in Electron Microscope of Cryptosporidium
Figure 1
A scanning electron micrograph of Cryptosporidium lining the intestinal tract.
(From: Gardiner et al., 1988, An Atlas of Protozoan Parasites in Animal Tissues, USDA Agriculture Handbook No. 651.)

Analysis of small subunit rRNA are able to separate the larger gastric (C. muris, C. serpentis Snakes, C. baileyi Birds) from the smaller intestinal species (C. parvum, C. felis, C. meleagridis, C. wrairi). C. meleagridis, C. felis and the dog genotype of C. parvum have been identified in stools of HIV – infected adults. C. parvum (Human, bovine), C. wrairi (Guinea pigs), C. muris (Bovine, murine), C. baileyi (Chickens), and C. serpentis (Snakes)

C. parvum is an intracellular protozoa parasite it was first describe in 1907 escribed as a cell – associated organism in the gastric mucosa of mice. It was recognized as a pathogen of mammals until 1971 when the infection was linked to calf diarrhea. Since the first reports of human cases in 1976 involved a 3 year old girl from rural Tennessee with severe gastroenteritis. After that, cryptosporidiosis has been found worldwide. Life cycle of Cryptosporidium shown in Figure 2.

Figure 2 : Life Cycle of Cryptosporidium.
Legend:

a. The infection is acquired through the ingestion of sporulated oocysts. Motile sporozoites emerge through an opening in the oocyst wall and attach to intestinal epithelial cells. This attachment and subsequent fusion of the microvilli are probably mediated by the rhoptries and micronemes found at the apical end of the sporozoite.

b. The sporozoite does not invade the epithelial cell, but induces the fusion of microvilli so that it becomes surrounded by a double membrane of host origin. This location is referred to as being 'extracytoplasmic'. The parasite undergoes a trophic period in which it probably derives nutrients from the host cell via an 'adhesion zone'.

c. The trophozoite undergoes an asexual replication, called either merogony or schizogony, resulting in the production of 4-8 merozoites.

d,e. Mature merozoites are released into the intestinal lumen and will infect new intestinal epithelial cells. Merozoites are morphologically similar to sporozoites and possess apical organelles. The merozoites can either undergo additional rounds of merogony, resulting in the production of more merozoites, or undergo a sexual cycle known as gametogony (or gamegony).

f. Some of the merozoites will develop into macrogamonts (also called macrogametocytes) which mature into macrogametes.

g. The microgamont (or microgametocyte) will undergo several rounds of replication producing numerous microgametes which are released into the intestinal lumen.

h. A microgamete will fuse with a macrogamete (still located in its extracytoplasmic position) and the resulting zygote undergoes sporogony.

i. Sporogony involves two rounds of replication resulting in four sporozoites. Fully sporulated oocysts are shed into the intestinal lumen at the completion of sporogony.

j,k. The infectious oocysts are excreted in the feces, thus completing the life cycle. An autoinfection in which excystation takes place within the same host may also be possible. It is proposed that the autoinfection is mediated by 'thin-walled' oocysts.

After ingestion by a new host → excystation and release of 4 sporozoites → disrupt microvilli and penetrate the enterocyte of the terminal ileum and jejunum, enveloping themselves in an extracytoplasmic compartment unique to Cryptosporidium. In the vacuole sporozoites mature to trophozoites → growth and maturation → 3 nuclear divisions as type I meronts – rupture – 6-8 merozoites → invade again → type I or type II meronts → release of macro & microgametocytes → fusion → zygote formation results in the development of 2 cyst population: thin-walled cysts (source of internal autoinfection) and thick-walled cyst, which are environmentally stable.

Molecular study have revealed two distinct genotype that there is a high phenotype variability between C.parvum genotype 1 and 2 at the level of gp 15. Genotype 1 has only been isolated from human source and is no infective for mice and calves. Genotype 2 has been isolated from both bovine and human source and is infective for mice and calves. These observations suggest that are two distinct transmission cycle: anthroponotic and zoonotic.3,7.

Method of Transmission:
Transmission of the 4-6 μm water-borne Cryptosporidium oocyst by fecal – oral route. ID50 of C.parvum genotype 2 is only 132 oocysts for seronegative
individuals seems to range from 1 to 1000 for three different genotype 2 isolates tested. Oocysts are fully sporulated, chlorine-resistant (can resist 3% chlorine for 18 hours) and can remain viable for about 18 months in a cool, damp or wet environment. Desiccation over a sufficient period of time (2 hours or more) is lethal to the oocysts Snap-freezing destroys oocysts reliably and a temperature of 65 degrees C inactivates them in 5-10 minutes. This organism is present in the faecal matter of infected humans and animals and is spread by the faecal-oral route. Transmission most often occurs through: person to person contact, particularly in farm lies and among small children (for example, in child care centres), drinking contaminated water, swimming in contaminated pools, food (in rare cases), handling infected animals, sexual activity that involves contact with faeces. A person most infectious when they have diarrhea, but the parasites may be excreted for several weeks after symptoms disappear. Oocysts in soils 27%, and in water 30% still viable at day 152.8 Cryptosporidiosis Outbreaks: There are separate transmission cycles for C. parvum genotype 1 and 2 and it seems to vary by geographic area. In the USA, Canada and Australia genotype 1 is the predominant type. In the UK genotype 2 is the cause of most sporadic cases. C. parvum is a well-known cause of water borne disease. This coccidian protozoan parasite may contaminate water from either human or zoonotic source. By flow cytometry enumeratinga small number of oocysts ( < 1,000 oocysts).9 Outbreaks of Cryptosporidiosis, have been reported in several countries. The most remarkable being a water borne outbreak in Milwaukee (Wisconsin) in 1993, that affected more than 400,000 people.10 On 1995, the Environmental Protection Agency (EPA) released some disturbing statistic: 53 million American drink water that violates US safety standards. Yearly, contaminated water may cause the death of nearly 1,000 people. Cryptosporidium warrant special attention, since there are three reason. First, it hard to detect, very few systems are tested specifically for cryptosporidium. And cryptosporidium can lurk in water supplies that have been labeled safe as result of acceptable coliform counts. Second, it is hard to get rid of cryptosporidium. Third, in severely immune-suppressed patient, cryptosporidium ia often untreatable.11 Clinical Manifestations: The clinical spectrum of cryptosporidiosis infections ranges from asymptomatic passage of oocyst to severe cholera-like gastroenteritis with biliary tract disease. The hallmark of cryptosporidial infection is diarrhea, which occurs after an incubation period of 2 to 14 days and is usually describe as watery voluminous, and occasionally explosive and foul-smelling. Additional symptoms are abdominal cramps, fatigue, anorexia, fever, nausea, and vomiting. These symptoms may lead to weight loss and dehydration.1,3 The first signs of the illness appear between 1 – 12 days (average 7 days) after a person becomes infected. In some cases there may be no symptoms at all. However, these people may still pass the disease on to others. Most healthy people recover in less than two weeks. People with a weak immune system may have more severe symptoms that can last for many weeks.1 In individuals in industrialized countries: latency period of 1 week and 9 days of diarrheal illness. In Children in tropical developing areas: similar illness with the
potency of relapse. In Immunocompromised host: self-limited infection (CD4 > 180 µL) or acute dehydrating diarrheal syndrome or a syndrome of chronic diarrhea and wasting or symptomatic involvement of the biliary and pancreatic ducts (C. parvum can induce apoptosis in cultured biliary and intestinal epithelia).

Cryptosporidiosis and AIDS:
In last 10 years, increasing numbers of cases have been reported. This rise reflects both an increased recognition of cryptosporidium as a human pathogen and the tremendous increase in the number of person with HIV infection. Patients with CD4+ T-cell counts below 200/mm³ frequently present with advanced disease and voluminous diarrhea with secondary wasting. In patients with CD4+ below 200, more likely to have diarrhea and other symptoms for long time. If CD4+ count above 200 and HIV-infected patients get cryptosporidiosis, patients may feel better in about one to three weeks, but might still have the infection and be able to pass it to others. If the patients still infected and CD4+ count later drops below 200, the cryptosporidiosis may act up again. In immunocompromised patients, an auto infection is also possible and this too many contribute to the increased severity. One study found that African-American AIDS patients in California indicate a significantly lower risk for presentation with cryptosporidiosis compare with whites.

In developing world, cryptosporidiosis has been diagnosed in up to 50 percent of AIDS patients with diarrhea. On 1999 – 2000 in Bangkok, 288 patients AIDS with diarrhea 19.2% had C.parvum and the rest other protozoa enteric infections. Control of HIV replication and immune deficiency by HAART (Highly Active Anti-retroviral Therapy) offers new therapeutic options in cryptosporidiosis and microsporidiosis and is crucial for the long-term prevention of opportunistic enteric infections or HIV-related malignancies.

Diagnosis:
The diagnosis of cryptosporidiosis is established by the identification of organism in stool or tissue specimens. The are most economically and commonly identified by modified acid fast staining. Stool organism may also be found by sucrose flotation testing. Stool blood or leucocyte are rare, and peripheral blood leucocytosis ar eosinophilia is uncommon. Immunoglobulin (IgM and IgG) may be measured but are not helpful in patients with AIDS with compromised serologic respons. Intestinal biopsy can be done in a certain case. The others diagnostic methods are DFA, ELISA, and PCR (more sensitive and less user dependent).

Treatment:
There is no therapeutic agent that eradicates intestinal cryptosporidiosis. In immunocompromised patients, Paromomycin was shown to decrease stool frequency and oocyst excretion. A combination of Paromomycin and Azithromycin or Roxithromycin also improved clinical symptoms. Most begin with initial dose 0f 500 mg Paromomycin four times per day orally for 14 – 30 days, and then prescribe a maintenance dose of 500 mg twice per day orally indefinitely. The long-term development of Paromomycin as a anticryptosporidial agent may be limited by its lack of absorption and inability to penetrate the biliary tract, a significant potential reservoir of cryptosporidiosis.
According to the current study recommended dose of Roxitrothromycin is 300 mg, twice a day for a minimum of five days to effectively treat the infection and to prevent resistance.\textsuperscript{17} Highly Active Antiretroviral Therapy (HAART) has an important role in the management of cryptosporidiosis.

**Complications:**
The biliary tract may serve as an important reservoir of chronic infection within the host, and also as a source of symptomatic disease. Biliary involvement occurs in approximately 15 percent of patients. When symptomatic patients develop signs and complaints of cholangitis with right upper quadrant pain, nausea, and vomiting. On laboratory testing, the alkaline phosphatase and gamma glutamyltransferase levels are elevated, serum bilirubin and transaminase levels may be abnormal. Radiographically, the gallbladder may appear thickened, and bile duct abnormalities may suggest sclerosing cholangitis. Pancreatitis may complicate biliary tract disease. Cryptosporidium has also been reported to cause respiratory tract disease. The significance of these reports is uncertain due to the frequent coexistence of other respiratory pathogens.\textsuperscript{12}

**Prevention and Treatment of Drinking Water:**
For home use Filtration Systems should be either able to remove particles that are 0.1-1 micrometers in size or filter water by reverse osmosis (0.001-0.01 microns, removes particles in the ionic range) or have an “absolute” 1-micron filter Municipal Systems. Micro-Filtration would provide ideal protection against water-borne outbreaks via Drinking Water. Strategies to protect the public water supplies in developed countries remains unclear.
References:

1. Fact Sheet: Cryptosporidiosis, Citation: NSW Public Health Bulletin, 2002; 12 (5): 142, From: URL: 

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