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A MINI-OVERVIEW ON ZOONOTIC CRYPTOSPORIDIOSIS

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Abstract

Cryptosporidium is an intracellular protozoan that is one of the most common parasitic enteric pathogens in humans. Infection is associated with gastrointestinal disease in sporadic self-limited outbreaks among immunocompetent hosts and chronic illness in immunosuppressed patients.

Transmission occurs via spread from an infected person or animal, or from a fecally contaminated environment, such as a food or water source. Cryptosporidiosis is associated with a secretory diarrhea and with malabsorption. The parasite intracellular nature interferes with intestinal absorption and secretion. Infection can be asymptomatic, a mild diarrheal illness, or severe enteritis with or without biliary tract involvement. In immunocompetent hosts, illness usually spontaneously resolves without therapy, but among immunosuppressed host infection can be a chronic debilitating illness with wasting and persistent diarrhea. Diagnosis depends on enzyme immunoassay or by microscopic identification of oocysts in stool or tissue. Organisms may be present in duodenal aspirates, bile secretions, biopsy specimens from gastrointestinal tract, or respiratory secretions.

Key words: Review, Cryptosporidiosis, Zoonosis, Transmission, Diagnosis, Egypt

Introduction

Cryptosporidium is an intracellular protozoan parasite associated with gastrointestinal diseases in all classes of vertebrates including mammals, reptiles, birds, and fish. Along with *Giardia*, it is among the most common parasitic enteric pathogens in humans. Organisms infect and reproduce in the epithelial cells of digestive or respiratory tracts. Infection was predominantly associated with diarrhea and biliary tract disease (Chen *et al*, 2002). Over the last few years molecular methods have enabled characterization and identification of species and genotypes within *Cryptosporidium* isolates (Robinson *et al*, 2008). There were about 20 species, including species that infect mammals, birds, reptiles and fish (Xiao *et al*, 2004). *C. parvum* (4 mcm diameter) was the main species responsible for clinical disease in humans (Fayer and Ungar, 1986). *C. parvum* was divided into two separate species: *C. hominis* (previously *C. parvum* genotype 1) and *C. parvum* (formerly *C. parvum* genotype 2). *C. hominis* apparently infects only man, while *C. parvum* is frequently found in many animals and in man (Morgan-Ryan *et al*, 2002).

Host range for many species seems to be quite variable and *C. felis, C. muris, C. canis, C. suis,* & *C. meleagridis* were identified in some persons (Cacciò, 2005). Also, heterogeneity within species may lead to variations in infectivity and clinical expression in different hosts (Tanriverdi *et al*, 2006).

Epidemiology: *Cryptosporidium* was first identified as a cause of gastrointestinal disease in humans in 1976, and is now recognized globally as an important cause of diarrhea in both children and adults (Yoder *et al*, 2012). *Cryptosporidium* species were identified in every continent except Antarctica (O'connor *et al*, 2011). *Cryptosporidium* was described as an etiologic agent in three main epidemiologic scenarios (Mor and Tzipori, 2008): a- Sporadic, often water -related outbreaks of self-limited diarrhea in immunocompetent hosts, b- Chronic, life-threatening illness in immunocompromised patients, particularly those with HIV infection, and c- Diarrhea and malnutrition in young children in developing countries The risk of severe and/or prolonged disease is increased in patients with cellular and humoral immune deficiencies, including HIV, organ transplanta-

tion, immunosuppressive drugs, IgA deficiency, and hypogammaglobulinemia. But, the number of cryptosporidiosis cases declined among HIV patients, largely due to immune reconstitution with highly active antiretroviral therapy (Le Moing *et al*, 1998).

Cryptosporidiosis was more common in countries that have increased crowding and poor sanitary conditions. In endemic areas, the incidence increases during rainy periods (Huang and White, 2006). It was also more frequent in children less than two years old, although outbreaks occur worldwide in all age groups (Mannheimer and Soave, 1994). The prevalence is increased in dairy farmers, which is probably because *C. parvum* caused diarrhea in cattle (Lengerich *et al*, 1993).

In the United States, cryptosporidiosis incidence was relatively stable from 2008 to 2010, following a greater than threefold increase from 2004 to 2007. Whether this is due to changing epidemiology or increased availability and application of diagnostic assays is unclear (CDC, 2012a). Although cryptosporidiosis affects persons in all age groups, cases are most frequently reported in children aged one to nine years in the United States. *Cryptosporidium* is present in 1 to 3% of immunocompetent patients with diarrhea in industrialized countries and 7 to 10% in developing countries (Jelinek *et al*, 1997). Seroprevalence rates are higher, being approximately 25 to 60% in the United States and 65 to 95% in some developing countries (Ungar *et al*, 1989).

The population-based laboratory surveillance data from Canada have shown *Cryptosporidium* sp infection to occur at an overall rate of 6.0/ 100,000 populations per year (Laupland and Church, 2005). The incidence was significantly higher in children than in adults (17.8/ 100,000 per year occurring among those aged <20 years of age, compared to 1.25/ 100,000/ year for adults ≥20 years of age [RR 14.29; 95% CI 9.77-21.11; $p<0.0001$]).

Cryptosporidiosis was a notified disease in the European Union, and surveillance data in Europe for 2005 showed 7960 cryptosporidiosis cases reported from 16 countries. A crude incidence rate was 1.9 cases per 100,000 overall, with considerable differences in cryptosporidiosis rates among countries (Semenza and Nichols, 2007). Prevalence was much higher in patients with HIV infection. In HIV-infected subjects in the United States and Europe, 8 to 30% and in developing countries 15 to 50% excreted oocysts, making it one of the commonest enteropathogens (Tarimo *et al*, 1996).

Transmission: A- Fecally passed *Cryptosporidium* oocysts are immediately infectious to those who ingest them. Transmission of cryptosporidiosis occurs via spread from an infected person or animal, or from a fecally contaminated environment such as a food or water source (CDC, 2007). Cryptosporidiosis outbreaks were associated with drinking water supplies, animal contact, travel, swimming pools, and recreational water facilities (CDC, 2008). The *C. hominis* infections were generally associated with foreign travel and in day-care associated cases, whereas *C. parvum* was associated with farm animal contact (Davies and Chalmers, 2009).

B- Ingestion of only a few oocysts (10 to 50) could lead to severe disease and persistent infection, particularly in immunodeficient patients. The ID_{50} for healthy people without serological evidence of previous cryptosporidiosis has been estimated at 132 oocysts for *C. parvum* (DuPont *et al*, 1995) and 10 to 83 oocysts for *C. hominis*; infected individuals can excrete up to a billion oocysts per infection (Chappell *et al*, 2006). Previous exposure and immunologic health also influence the host susceptibility.

C- A major source of infection is contaminated drinking or swimming water, which causes community outbreaks and travelers' diarrhea. *Cryptosporidium* was present in 2.8% of 795 international travelers with diarrhea in one report (Jokipii *et al*, 1985). *Cryptosporidium* oocysts are present in 65 to 97% of surface waters and are difficult to eradicate since oocysts are resistant to many

disinfectants, are not effectively removed by many filtration systems, and can survive in the environment for months. As a result, oocysts can be intermittently detected in tap water (Juranek, 1995). So, swimming pools, natural ponds, and other recreational water sources are significant sources of infection (CDC, 2009). An outbreak of cryptosporidiosis was reported among firefighters responding to a fire in a barn housing calves and numerous waterborne outbreaks were reported (CDC, 2012). The largest occurred in 1993, when 403,000 residents of Milwaukee developed gastrointestinal sym-ptoms after their drinking water was contaminated (Levy *et al*, 1998). Extension of outbreak might actually have been underappreciated; antibody determinations to two *C. parvum* antigens were made in children 6 months to 12 years of age who had routine lead screening performed during March to May of 1993 (McDonald, *et al*, 2001). The prevalence of antibodies during a five-week period rose from 15 to 82% &17 to 87% in two southern zip codes in the city, which were close to the implicated water treatment plant. Outbreaks associated with apple cider contaminated by the Cryptosporidia oocysts were also reported (Causer *et al*, 2006). An outbreak associated with a local waterpark involving more than 350 cases was reported from Illinois in 2001 (Pönka *et al*, 2009). Generally speaking, oocysts of *Cryptosporidium* occur in the aquatic environment throughout the world (Frost *et al*, 1998). They were found in most surface waters, where their concentration was related to the level of faecal pollution or human use of the water (LeChevallier *et al*, 1991). Environmentally robust oocysts were very persistent in water (Rabold *et al*, 1994; Chauret *et al*, 1995) and extremely resistant to disinfectants commonly used in drinking-water treatment (Gallaher *et al*, 1989).

In most tropical countries, transmission in children is usually associated with the rainy season, and waterborne transmission is considered a major route in epidemiology of cryptosporidiosis in these areas (Peng *et al*, 2003). Avoid water that might be contaminated, as commercially bottled water, water previously boiled for a minute and left to cool and at elevations above 6,500 feet (1,981meters), boil for 3 minutes. Also, use a filter designed to remove *Cryptosporidium*. Label might read 'NSF 53' or 'NSF 58'.

D- Foodborne transmission: Foodborne outbreaks are less common than waterborne outbreaks but are reported, usually in the setting of contaminated cafeteria food (Ethelberg *et al*, 2009). In an outbreak associated with consumption of food in a university cafeteria linked a *C. parvum* genotype 1 (now *C. hominis*) isolate to an infected food handler who prepared raw produce. Eighty-eight students and four employees became ill; *C. hominis* was isolated from 16 of 23 sick students (70%) and two of four employees (Quiroz *et al*, 2000).

E- Person-to-person transmission is common particularly among household members, sexual partners, children in daycare centers and their caretakers, and healthcare workers (Musher and Musher, 2004). In one study, 19% of household contacts of index cases of cryptosporidiosis developed acute infection. Also, 27% of asymptomatic children attending a day care center in New York excreted oocysts (Crawford *et al*, 1988). Numerous waterborne outbreaks of cryptosporidiosis have occurred in the United States, Canada, United Kingdom, France, Australia, Japan, and other industrialized nations (Yamamoto *et al*, 2000; Dalle *et al*, 2003).

F- Respiratory transmission: In Uganda, 1156 children who presented with diarrhea, 926 fecal samples were screened, 116 (13%) of which were positive for cryptosporidium. Among the patients who had evidence of fecal infection, 48 also had testing of sputum samples; 17 (35%) were positive for cryptosporidium. Vast majority of children with respiratory cryptosporidiosis were HIV-seronegative (94%). Transmission might arise if oocysts were aerosolized during coughing; but, whether or not respiratory transmission

actually occurred was unknown (Mor *et al*, 2010). Højlyng *et al*. (1987**)** reported a child of airborne cryptosporidiosis transmission.

Respiratory and intestinal cryptosporidiosis in birds was attributed to *C. baileyi*, *C. meleagridis*, *C. serpentis* infects reptiles and *C. nasorum* infected fish (Fayer *et al*, 1997; Koudela and Modry, 1998; Lindsay *et al*, 2000).

Pathogenesis of cryptosporidiosis is not well understood. The organisms cause a secretory diarrhea that can be associated with malabsorption. The intracellular nature of the infection interferes with intestinal absorption and secretion. The organisms can spread via the intestinal lumen to involve the biliary system, where they can cause stricturing and cholangitis. No specific toxin was identified, although one study of young children in Haiti reported the increased systemic and intestinal proinflammatory cytokines (e.g., TNF & interleukin-8) compared to healthy controls (Kirkpatrick *et al*, 2006).

Cryptosporidia are found within epithelial cells associated with distortion of the villus architecture. Inflammatory changes may be present (Heyworth, 1996). Progressive morphological and functional abnormalities of the small intestine occur as parasite numbers increase, although intensity of infection and inflammation did not correlate well with the severity of clinical disease. Whether differences in the organism's virulence or the level of host immunity primarily account for the variable course of infection in different people was not yet well understood (Genta *et al*, 1993).

The immune response associated with cryptosporidiosis involves cellular and humoral components. T-lymphocyte cellular responses are important in controlling infection, as evidenced by the increased disease severity in HIV-infected patients with CD4 counts less than 100 cells/microL.

Specific IgM, IgG, and/or IgA responses develop during infection. Epidemiologic evidence for protective immunity to *Cryptosporidium* has been suggested by the observation that residents in areas where cryptosporidiosis is endemic have milder symptoms with subsequent infections (Okhuysen *et al*, 1998). But, the development of antibodies is not necessarily associated with clearance of infection, as illustrated in studies of HIV-infected patients, who developed serum and intestinal antibodies but failed to clear the infection (Benhamou *et al*, 1995). The production of IFN-gamma is involved in the resolution of infection (Culshaw *et al*, 1997).

The life cycle of *Cryptosporidium* can be completed within a single host. Oocysts are ingested, undergo excystation in the small bowel, and release four banana-shaped motile sporozoites that attach to the epithelial cell wall. Sporozoites mature asexually into meronts, which release merozoites intraluminally. These can reinvade host cells, resulting in autoinfection, or can undergo sexual maturation to form new oocytes which can excyst within host gastrointestinal tract or can pass out into environment. Oocysts are infectious and remain viable for many months at a wide range of temperatures.

Clinical manifestations: Cryptosporidiosis can cause an asymptomatic infection, a mild diarrheal illness, or severe enteritis with or without biliary tract involvement. Asymptomatic infection can occur in immunocompetent and immunodeficient patients. About 30% of childhood infections were asymptomatic (Janoff *et al*, 1990). Infection in elderly patients led to severe volume depletion in association with high case-fatality rates. The incubation period is usually 7 to 10 days (range 3 to 28 days). Number of oocysts ingested appeared to be related to the time to and duration of infection, but not the severity of illness (Mor *et al*, 2009).

Patients who develop diarrhea frequently have associated malaise, nausea and anorexia, crampy abdominal pain, and low-grade fever. Diarrhea may be acute or chronic, transient, intermittent or continuous, and scant or voluminous with up to 25L/day of watery stool. Fecal blood or leukocytes are

rare unless co-infected with another enteric pathogen. Patients with chronic diarrhea can develop profound weight loss.

The illness usually resolves without therapy in 10 to 14 days in immunologically healthy people, although it persisted longer or relapsed after initial improvement. Excretion of oocysts after resolution of clinical symptoms continued for prolonged periods (Jokipii and Jokipii, 1986). There was some evidence in non-immunocompromised infants that Cryptosporidia infection led to persistent diarrhea with a lasted adverse effect on nutritional status and growth (Checkley *et al*, 1998).

In immunocompromised hosts (particularly those with T-cell immunodeficiency), the illness was more frequently protracted and severe, and led to significant wasting, particularly when the CD4=<100 cells/microL. The specific species or subtype families were associated with different clinical manifestations. In a cross-sectional study of 230 HIV-infected patients in Peru, infection with *C. hominis* was associated with diarrhea alone while infection with *C. parvum* was associated with diarrhea and vomiting (Cama *et al*, 2007). The American Gastroenterological Association (AGA) technical reviewed the malnutrition and cachexia, chronic diarrhea, and hepatobiliary diseases in patients with HIV (AGA, 1996).

Other clinical manifestations in AIDS patients included cholecystitis, cholangitis, hepatitis, pancreatitis, and respiratory tract involvement. Biliary tract involvement affects 10-30% of patients with AIDS and result in acalculous cholecystitis, sclerosing cholangitis, pancreatitis, right upper quadrant pain and fever (Soave and Johnson, 1988).

AIDS cholangiopathy is a syndrome of biliary obstruction due to infection-related to biliary tract strictures (Chen and LaRusso, 2002). Organism commonly associated was *C. parvum*; others were *Micros-poridium*, cytomegalovirus (CMV), and *Cyclospora cayetanensis* (Teixidor *et al*, 1991). Involvement of large intrahepatic ducts was usually associated with *C. parvum* and CMV infection (Benhamou *et al*, 1993).

Pulmonary involvement was described, but, unclear whether organism was a true pathogen or merely colonizes the respiratory tract (Moore and Frenkel, 1991). Nonspecific respiratory symptoms as cough were reported but not disseminated cryptosporidiosis (Meynard *et al*, 1996).

Laboratory abnormalities: The presence of laboratory abnormalities depends upon the severity and duration of infection. Serum alkaline phosphatase may be elevated in patients with biliary tract involvement. In such patients, ultrasound and CT imaging may show an enlarged gallbladder with a thickened wall and dilated intra- and extrahepatic biliary ducts. Diagnosis of biliary involvement is confirmed by histology or by examination of bile for oocysts, since stool specimens may or may not be positive. Diagnosis of AIDS cholangiopathy is usually made by endoscopic retrograde cholangiopancreatography (ERCP). But, for early evaluation and in selecting patients for ERCP, Ultrasound proved the most cost-effective initial study, with sensitivity ranged from 75-97% & specificity up to 100% (Bouche *et al*, 1993).

Patients with severe, protracted disease can have evidence of malabsorption. (Malabsorption refers to impaired absorption of nutrients. It may be congenital defects in the membrane transport systems of the small intestinal epithelium or from acquired defects in the epithelial absorptive surface, as well as maldigestion due to impaired digestion of nutrients within the intestinal lumen or at the terminal digestive site of brush border membrane of mucosal epithelial cells (Hogenauer and Hammer, 2010). Global malabsorption results from so many diseases associated with either diffuse mucosal involvement or a reduced absorptive surface. Disorders of malabsorption lead to decreased iron absorption and produce iron deficiency anemia (Saboor *et al*, 2015).

Diagnosis: The diagnosis of cryptosporidiosis is made by microscopic identification of

the oocysts in stool or tissue. The organisms may also be present in duodenal aspirates, bile secretions, biopsy specimens from affected gastrointestinal tissue, or respiratory secretions. Almost all active Cryptosporidium infections are diagnosed by analysis of stool specimens. Examination of intestinal or biliary biopsy is sometimes used in the diagnosis of cryptosporidiosis in AIDS patients (Clayton *et al,* 1994).

Microscopically *Cryptosporidium* species cannot be cultivated in vitro. As a result, the diagnosis is primarily based on microscopic identification. The laboratory should be alerted to the potential diagnosis and specific stains for the organisms should be requested, since routine examination for ova and parasites usually does not detect cryptosporidia spores. Specimens can be examined fresh or formalin-fixed, by light or phase-contrast microscopy. Modified acid-fast stains are usually used, although the organisms also can be seen using H & E, Giemsa, or malachite green staining. With modified acid-fast stain, oocysts stain red or pink and 4 to 6 mcm in diameter. Light microscopy is unable to distinguish between genetically distinct parasites.

Accuracy of the acid-fast stain has not been well established but depends in part upon number of stool specimens examined, since oocysts number shed in feces was not constant. An examination of a single stool specimen identified 30% of intestinal cryptosporidiosis (Blanshard *et al*, 1992).

Number of specimens required to conclusively exclude the diagnosis has not been studied, but in chronic infections, examining up to three specimens is reasonable. Also, examination of unformed specimens and concentrated specimens increase the diagnostic yield. Fecal specimens usually lack leukocytes and erythrocytes.

Histopathology: Cryptosporidial enteritis can be diagnosed from H & E staining; parasite appears basophilic and occurs either alone or in clusters on the brush border of the mucosal surface. Because infection can be patchy, biopsy specimens may be less sensitive than stool examination.

Monoclonal antibodies and enzyme immunoassays: Monoclonal antibodies against the oocyst wall and antigen capture ELISA tests have been used in fluorescent assays (e.g., Meridian Merifluor assay and the TechLab Crypto IF kit) to detect Cryptosporidium in fecal specimens or in tissue specimens. These techniques increased the sensitivity compared to routine light microscopy and are easy to perform (Kehl *et al,* 1995). Enzyme immunoassay kits include the Alexon ProSpect Assay, the Seradyn ColorVue and the Meridian Premier *Cryptosporidium*, which have been evaluated in a number of studies: 1- ProSpect T kit had a sensitivity of 100% and specificity of 99% when compared to modified acid-fast stain (Parisi and Tierno, 1995) 99%, respectively, and specificities of 100 for both when compared to Meridian Merifluor assay as a reference test (Garcia and Shimizu, 1997). 3- ProSpecT and Color Vue had sensitivities of 96 & 76%, & specificities of 98 & 100%, respectively, by using Merifluor stain as a reference test (Aarnaes *et al*, 1994). 4- Acid-fast stain & EIA had sensitivities of 94 & 100%, and specificities of 76 & 100%, respectively, compared to direct immunofluorescence (Siddons *et al*, 1992).

Advantages of the ELISA tests are that they are easy to use, are not affected by preservatives, and do not require the degree of technical skill needed for microscopy (Dagan *et al*, 1996). But, a major disadvantage is their high cost. Faulty ProSpecT kits were associated with false positive results (Doing *et al*, 1999).

PCR: Although diagnosis of cryptosporidiosis is generally based on microscopy, this method offers no information on the infecting species, which can be helpful in epidemiologic investigations. PCR testing has the ability to differentiate between *Cryptosporidium* genotypes, thereby having a potential use in detecting the source of outbreaks (Morgan *et al,* 1998).

A commercially available PCR-ELISA-based test also allows for detection and genotyping of cryptosporidium in the biological samples. A study of this hybrid assay in 33 stool samples showed a sensitivity and specificity of 97 & 100%, respectively (Savin *et al*, 2008). Serologic detection using the IFA assays or ELISA tests are available, but used only as an epidemiologic tool, because antibody persistence limits the usefulness in diagnosis of acute infection.

Treatment: Most people with healthy immune systems recover without treatment. Diarrhea can be managed by drinking plenty of fluids to prevent dehydration. People with poor health or with weakened immune systems are at higher risk for more severe and prolonged illness. Young children and pregnant women may be more susceptible to dehydration due to diarrhea and should drink plenty of fluids while ill. Rapid loss of fluids from diarrhea may be especially life threatening to babies. So, parents should talk to their healthcare providers about fluid replacement therapy options for infants.

Anti-diarrheal medicine may help slow down diarrhea, but a healthcare provider must be consulted. Nitazoxanide was FDA-approved for the treatment of diarrhea caused by cryptosporidiosis in people with healthy immune systems and is available by prescription. But, effectiveness of nitazoxanide® in immunosuppressed people was not proved.

The HIV-positive individuals who were suspect they have cryptosporidiosis should contact their healthcare provider. For those individuals with AIDS, anti-retroviral therapy that improves the immune status would also decrease or eliminates the cryptosporidiosis symptoms. Nevertheless, even if the symptoms disappeared, the cryptosporidiosis was often not curable and symptoms might return if the immune status worsens (CDC, 2015).

In Egypt, Youssef *et al*. (2008) reviewed 61 published papers between 1985 & 2006. Nineteen studies examined immunocompetent individuals with diarrhea presented to inpatient or outpatient clinics with a *C. parvum* prevalence ranged from 0%-47% (median 9%, IQR 3-15%). Then, many papers were published on zoonotic cryptosporidiosis (Feng *et al*, 2018). A multicenter, randomized, double-blind, placebo-controlled trial was conducted in 90 outpatients 12 years of age and older They were assigned to receive 500mg of Nitazoxanide® (tablet or suspension) or placebo, given twice daily for three days and clinically improved at 4th day post-treament (Rossignol *et al*, 2006). *C. parvum* infected mice treated with Ginger, Mirazid®, Garlic and/or Metronidazole® showed complete oocysts elimination 9th day post-infection. Garlic successfully eradicated oocysts of mice from stool and intestine. Ginger supplementation to infected mice markedly corrected elevation in inflammatory risk factors and implied its potential antioxidant, anti-inflammatory and immunomodulatory capabilities (Abou-el-Nour *et al*, 2016).

Conclusion

Evidence showed a high global burden of cryptosporidiosis, mainly among children & immunocompromised or malnourished people. Microscopy and antigen assays are useful for clinical diagnosis at genus level. Species and subtyping identification are important for outbreaks, epidemiology, burden assessment, and transmission risk. Efforts to develop vaccine are limited by insufficient understanding of the immune responses mediating protection

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