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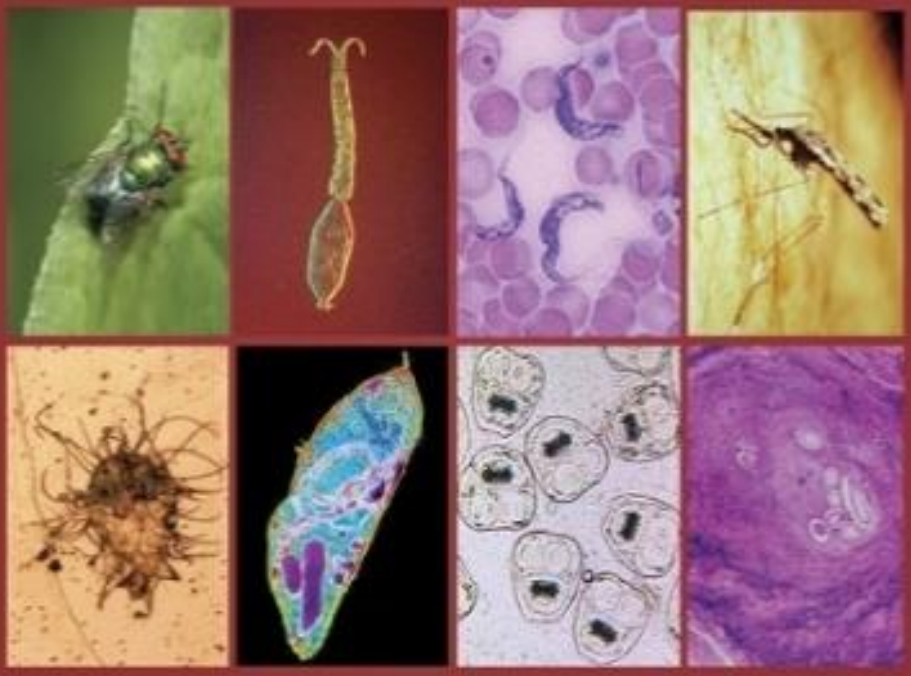

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Review Article on Cryptosporidiosis: Epidemiology, Clinical Presentation, Diagnosis, and Treatment

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ABSTRACT

Cryptosporidium is the causative agent of Cryptosporidiosis, they are apicomplexan protozoan parasites that have the ability to reproduce asexually and sexually to infect the micro-villous border of the gastrointestinal epithelium causing self-limited diarrhea in immune-competent and severe life-threatening diarrhea in immune-compromised individuals worldwide and may encounter fatal complications (Mohammed *et al*, 2017).

Cryptosporidium species are one of the most important waterborne pathogens worldwide, they have been ranked as the sixth most important foodborne parasitic infection of humans and domestic animals and a leading cause of mortality from waterborne gastrointestinal diseases, which can be, transmitted from animals, human to human, water, food, and tends to cause waterborne outbreaks (Zueter *et al*, 2019).

INTRODUCTION

Cryptosporidium spp. are intracellular parasites of special concern, especially in Mediterranean countries with limited resources and low socioeconomic status (Janssen and Snowden, 2022). Cryptosporidiosis is an extensively worldwide spread protozoan caused by several species, but C. *parvum* and C. hominis are the commonest zoonotic ones (Ježková *et al*, 2021). Cryptosporidiosis is one of the most widespread emerging zoonotic diseases with reservoirs in cattle, and domestic animals parasitic disease. It represents a major global health problem worldwide, particularly among immunocompromised individuals and children; representing the second major cause of diarrhea and death in children after the rotavirus. (Janssen and Snowden, 2022).

Ernest Edward Tyzzer first described the genus Cryptosporidium, between 1907 and 1912, they reported the identification of novel coccidia-like organisms in the gastric glands (Tyzzer, 1907 and Tyzzer, 1910) or small intestine (Tyzzer, 1912) of laboratory mice. Human cryptosporidiosis was first reported in 1976; after that, it was identified as the major cause of chronic diarrhea in HIV-positive patients and children and as a cause of zoonotic and waterborne diarrheal outbreaks (Xiao *et al*, 2004).

Transmission: Cryptosporidiosis transmitted via ingestion of small-sized oocysts (4-6 μm) in contaminated food or water and through direct person-to-person or animal-to-person contact, oocysts can be transmitted by the inhalation of aerosolized droplets which are one of the risk factors in the rapid transmission of *Cryptosporidium* oocysts, it can spread through coughing and sneezing (Pane and Putignani, 2022).

Oocysts are highly resistant to common household disinfectants; it is considered to be one of the causes of waterborne diarrheal outbreaks; oocysts may remain viable in water for over 140 days. A single oocyst is sufficient to produce infection before being shed in the stool and transmitted to another host via the faecal-oral route and causing disease in susceptible hosts. *Cryptosporidium* is also responsible for travelers' diarrhea, its small size, low infectious dose, high chlorine resistance and zoonotic potential, which play a major role in the transmission of infection (Dillingham *et al*, 2002; Gururajan *et al*, 2021).

Cryptosporidium species have a complex life cycle, they can grow only in living cells but can survive in the environment for long periods without losing their infectivity and requires a single host. (CDC, 2021).

Epidemiology:

During the last quarter of the 20th century and at the beginning of the 21st century, Cryptosporidiosis has caused a million deaths from gastroenteritis with high mortality rates in children under five years of age.

Risk factors of *Cryptosporidium* infection include immune-compromised individuals, children, people in contact with animals, poor social infrastructures, contaminated water supply by sewage, contamination of vegetables and fruits with animal manure or by the usage of animal manure as fertilizer in agriculture (Ramadan *et al*, 2020), lack of water treatment, sanitation problems, overcrowding, especially rural areas in developing countries and exposure to human immunodeficiency virus-infected family members (Kooh *et al*, 2020).

Cryptosporidium are more than 44 species, and more than 120 genotypes have been found, including *C. hominis* and *C. parvum*, which represent the 95% of cryptosporidiosis cases in humans. A few cases of C. meleagridis infections have also been reported in humans, as well as *C. canis, C. felis, C. suis, C. muris,* and *C. andersoni* in immunocompromised individuals. C. *parvum*, is responsible for infection in multiple hosts, such as mammals, birds, reptiles, and fish. *C. meleagridis, C. felis, C. canis, C. ubiquitum, C. cuniculus, C. muris, C. andersoni, C. erinacei, C. tyzzeri, C. bovis, C. suis, C. scrofarum, C. occultus, C. xiaoi, C. fayeri, C. ditrichi, C. viatorum*, and other species are mostly reported in animal infections (CDC, 2022).

Immune Response to Cryptosporidiosis:

The host immune response to *Cryptosporidium* infection involves components of both the innate and adaptive immune systems; both mechanisms play a role in protection against Cryptosporidiosis. Intestinal epithelial cells are the first line of defense, followed by the recruitment of innate immune cells such as NK cells, dendritic cells, macrophages and mast cells (Ludington and Ward, 2015).

Cell-mediated immunity is an important cornerstone of the immune response to infection and diagnosis is mainly based on the identification of oocysts from fecal materials (CDC, 2021). Intestinal epithelial cells are an important component of gastrointestinal mucosal immunity; they protect the intestinal mucosa from commensal microbes or the invasion of pathogenic organisms. In addition, they play a critical role in the initiation, regulation, and resolution of both innate and adaptive immune reactions against *Cryptosporidium* infection due to the nature of the parasitophorous vacuole produced by *Cryptosporidium* in infected host cells and releasing antimicrobial peptides. Also, inflammatory chemokines and cytokines activate immune effector cells to the infection sites, nitric oxide which can kill and inhibit C. *parvum* growth. (He *et al*, 2021).

Immune evasion mechanism of cryptosporidiosis within infected epithelial cells through activation of NF-κB signaling

to activate antiapoptotic cell death signaling in infected cells. It can cause the depletion of the signal transducer and activator of transcription 1α (STAT1α), a critical transcription factor in IFN-γ signaling, resulting in the suppression of IFN-γ-dependent gene transactivation in the intestinal epithelium. The infection of host epithelial cells suppresses the expression of the C-C motif chemokine ligand 20 (CCL20), a cytokine with anti-parasitic capacity (Borad and Ward, 2010).

The immunological response of cryptosporidiosis depends on CD3+ / CD4+ lymphocytes for recovery from the infection. Upon antigen stimulation, CD4+ T cells play an important role via secretion of cytokines such as IL-2, IFN-α, IFN-γ secreted by Th1 cells, and IL-4, IL-6 secreted by Th2 cells, IFN-γ also resists the invasion of *Cryptosporidium* pathogens (Atia *et al*, 2021). During the acute phase of infection, sporozoites stimulate macrophages and dendritic cells to secrete IL-12 that act with IL-18 and TNF-α to activate natural killer (NK) cells. In addition, TNF-α prevents *Cryptosporidium* infection in enterocytes, and other proinflammatory cytokines (IL-1, IL-6) released by multiple immunocompetent cells have a protective effect. This suggests a major role for host immune factors in controlling cryptosporidiosis (Barakat *et al*, 2009).

Pathophysiology:

Cryptosporidium oocysts undergo excystation and release infective sporozoites; the released sporozoite then attaches to the apical membrane of intestinal epithelial cells and forms an intracellular vacuole in which the parasite develops. The parasites undergo asexual multiplication (schizogony or merogony) within extracytoplasmic parasitophorous vacuoles and then sexual multiplication (gametogony) producing microgamonts (male) and macrogamonts (female). infection can be spread to extraintestinal sites in immunodeficient hosts (Janssen and Snowden, 2022).

Invasion of host cells is restricted to the luminal border of the enterocytes and leads to displacement of the microvillous border and loss of the surface epithelium, causing changes in the villous architecture with villous atrophy, blunting and crypt cell hyperplasia and mixed inflammatory cell infiltration in the lamina propria (Chen *et al*., 2002 and Chalmers & Davies, 2010).

Cryptosporidiosis is caused by three mechanisms; infiltration of the lamina propria by inflammatory cells, increased epithelial permeability, villous atrophy, and cell death, and malabsorption due to loss of intestinal architecture. The incubation period ranges from two to ten days, with an average of seven days.

Each cell reproduces in massive quantities, producing both thick and thin-walled oocysts that cause auto-infection which is responsible for the increase in the severity of disease seen in immunocompromised patients (Janssen and Snowden, 2022).

Cryptosporidiosis Prevalence:

Cryptosporidiosis considered being the riskiest opportunistic infection for patients with acquired immunodeficiency syndrome and among malnourished children. Globally, it has been ranked as the sixth most important foodborne parasitic infection of humans and domestic animals (Zueter, 2020).

The global prevalence of *Cryptosporidium* infection was 7.6 % with the highest prevalence in Mexico, Nigeria, Bangladesh and the Republic of Korea respectively among immunocompetent and immunocompromised people from low-income countries, people with gastrointestinal symptoms, people younger than 5 years old and residents living in rural areas (Dong *et al*, 2020).

The cryptosporidiosis death rate among young children less than 5 years old is about 30–50% of the worldwide, it is reported to be the second cause of death from diarrhea after rotavirus, in Sub Saharan Africa about 2.9 million deaths every year in children aged less than 2 years

old from cryptospordosis (Tombang *et al*, 2019).

The broad range in prevalence may be due to many factors such as the immune state of the host, the patient's age, and environmental habitats. Food is grown in soil fertilized with manure considered a potential source of infection and, contaminated water represents the major source of infections for humans (Walter *et al*, 2021).

Clinical Pictures:

Cryptosporidiosis is an opportunistic pathogen that presents as profuse watery diarrhea "cholera-like" and wasting due to malabsorption due to loss of intestinal architecture. In immunocompetent patients, infection is usually limited to the small intestine and diarrhea resolves spontaneously within seven to 14 days. In infants; the symptoms are abdominal pain, fever, nausea, vomiting, nutritional defects, elevated levels of lactoferrin and immune system-related defects. (Omolabi *et al*, 2022).

In immunocompromised persons, the infection is severe, life-threatening gastrointestinal infection and disseminate to other organs such as the pancreas, biliary duct, and respiratory tract. Oocysts can be detected in the sputum and bronchial aspirates of children with intestinal cryptosporidiosis and cough (Atia *et al*, 2021).

Complications:

Cryptosporidiosis includes severe dehydration,electrolyte imbalance, malnu trition, growth delays, and cognitive impairment, biliary involvement with sclerosing cholangitis, calculous cholecystitis, papillary stenosis, pancreatitis, low CD4 counts and pulmonary complications can develop in individuals with AIDS and in transplant recipients, (Korpe *et al*, 2016). Malnutrition increases the risk of infection, it is associated with more chronic symptoms and higher mortality rates (O'connor *et al*, 2011; Janssen and Snowden, 2022).

Diagnosis:

Cryptosporidium infection can be diagnosed by different methods including conventional microscopic techniques by wet mount or stained smears with modified acid-fast stain or by fluorescent stains. A single stool specimen is usually adequate for the diagnosis of cryptosporidiosis in patients with profuse diarrhea while repeating microscopic examination of three samples collected on alternating days is considered ideal due to the shedding of oocysts intermittently. Immunological detection of antigens or antibodies, histological examination of the biopsy and various molecular methods for the detection of DNA are also available (Smith, 2007). (Bayoumy *et al*, 2010).

The acid-fast stain has an advantage; low cost, no need for special microscopes and the ability to detect other pathogens such as Isospora and Cyclospora (Casemore *et al*., 1985 and shahina *et al*; 2011), on the other hand, it has a disadvantage; is time-consuming, and requires skillful experience for detection of *Cryptosporidium* oocyst, and the specimen must be handled in a safety cabinet as it is classified as a biosafety level II organism (Garcia *et al*, 2018; Razakandrainibe *et al*, 2021).

Immunological-based detection methods, such as Enzyme-Linked Immunosorbent Assay ELISA is gaining popularity as an immunology-based technique for the diagnosis of cryptosporidiosis is simple and rapid and offers a less subjective method than microscopy for detecting this protozoan in faecal samples. It is characterized by high sensitivity and specificity, even in the presence of very low quantities of antigen. It is also a useful assay for ruling out cryptosporidiosis in immunocompromised individuals, especially when there are indicative clinical signs with inconclusive microscopic diagnosis, or in large-scale epidemiological surveys (Marques *et al*., 2005; Vohra *et al*; 2012).

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Nahas *et al*, (2022) evaluated the sensitivity, specificity and usefulness of immunological methods; (ICT & ELISA) to detect *Cryptosporidium* from fecal specimens, they reported that immunoassay techniques are standardized test, requires minimal training and are easier to perform, with high sensitivity and specificity for routine diagnosis, simple, rapid, reliable methods for cryptosporidiosis diagnosis. The superiority of immunological diagnostic tests over the traditional microscopic methods due to resembling symptoms of gastrointestinal infections and a little number of diagnostic stages in chronic cases and its importance in epidemiological studies.

Histological examination of the biopsy of human gastrointestinal mucosa by identification of the intracellular stages stained with haematoxylin and eosin sections, the parasite appears as small, spherical, basophilic bodies (2-5µm) within the microvillous region of the intestinal mucosa (Clark, 1999 and Vohra *et al*; 2012).

Molecular methods:

Polymerase Chain Reaction (PCR) technology provides specific diagnosis to the species level with high sensitivity. Detection of cryptosporidiosis using PCR-based methods is more sensitive than conventional microscopical and serological methods for detecting oocysts in faeces. Molecular methods can also identify the species/genotypes and subtypes to determine the epidemiology of *Cryptosporidium* and predict transmission routes (Caccio *et al*., 2005 and Ware *et al*., 2013).

The diagnosis and genetic characterization of the different species of *Cryptosporidium* are essential for the prevention, surveillance, and control of cryptosporidiosis (Pacheco *et al*, 2022).

Treatment:

People with healthy immune systems will recover without treatment. Diarrhea can be managed by drinking plenty of fluids to prevent dehydration. The most important aspect of treatment is fluid and electrolyte replacement with oral rehydration solutions, although intravenous therapy may be necessary and nutritional support is probably beneficial (Zintl *et al*; 2009).

People who are in poor health or who have weakened immune systems are at higher risk for more severe and prolonged illness. Young children and pregnant women may be more susceptible to dehydration resulting from diarrhea and should drink plenty of fluids during illness. Rapid loss of fluids from diarrhea may be especially life-threatening to babies. Anti-diarrheal medicine may help slow down diarrhea (CDC, 2012)

Nitazoxanide has been approved by Food & Drug Administration (FDA) for the treatment of diarrhea caused by *Cryptosporidium* in people with healthy immune systems and is available by prescription (CDC, 2012). Before the approval of nitazoxanide in November 2002, no drug for the treatment of cryptosporidiosis had been approved. It is a broad-spectrum antiparasitic salicylamide derivative of nitrothiazole. It reduces the duration of diarrhoea and oocyst shedding in both, IC and IS patients (Singh, *et al*, 2011).

For HIV-positive individuals who suspect they have cryptosporidiosis highly active antiretroviral therapy (HAART) that improves the immune status will also decrease or eliminate symptoms of cryptosporidiosis. However, even if symptoms disappear, cryptosporidiosis is often not curable and the symptoms may return if the immune status gets worsen (Ahmadpour *et al*, 2020).

The search for bioactive plants which can be used as nonconventional anti-parasitic treatment has received considerable attention in recent times because of the increasing worldwide development of resistance to chemical drugs in parasitic infections (Abouel-Nour, et *al*, 2016).

Prevention:

Patients with diarrhea should wash their hands thoroughly and avoid using public swimming pools during their illness for at least 2 weeks after diarrhea to prevent the spreading of the disease. In order to prevent fecal-oral spread instruct HIV patients, daycare workers, food handlers, and healthcare workers to wear gloves and wash their hands thoroughly after contact with human feces as changing diapers (CDC.2021).

Immunocompromised patients, particularly HIV patients who have fewer than 200 CD4 cells/µL should consider either using 1-µm water filters when drinking tap water or using boiled drinking water (CDC.2021).

Organ transplanted recipients and AIDS patients should avoid contact with newborn animals including domestic animals and patients with diarrhea, also they have to avoid communal recreational water such as public swimming pools. New pets for patients with AIDS should be older than 6 months and should not have diarrhea (CDC.2021)

Recommendations:

- In Egypt, there is a scarcity of studies on *Cryptosporidium* species, so more research is recommended to display the actual state of this infection in Egypt.
- Encourage more studies using different doses and different regimens of natural therapies and new drug regimens that may replace the standard chemotherapeutic drugs that are used for the treatment of cryptosporidiosis.
- In addition to the routine investigations for immunocompromized patients, clinicians should also request laboratory investigations for *Cryptosporidium*.
- Serological screening for cryptosporidiosis should be conducted in high-risk persons.
- The emergence of alternative chemotherapeutic drugs for nitazoxanide should be considered.
- Perform local and national surveillance, prevalence and progress in drug and vaccine trials.

Conclusion:

Cryptosporidiosis represents one of the major global health problems worldwide, particularly among immunocompromised individuals and children. Its prevalence varies widely and this broad range in prevalence may be attributed to many factors such as the immune state of the patients, the patient's age, environmental habitats, food grown in soil fertilized with manure considered a potential source of infection and, contaminated water represents the major source of infections for humans.

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