Cryptosporidiosis in Calves: Clinical Implications, Virulence Factors, and Future Prospectives with Special Reference to Egypt Situation

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ABSTRACT
Cryptosporidiosis, an enteric disease of cattle particularly in young calves is becoming increasingly important. It is a common and significant zoonotic gastrointestinal parasite and a highly infectious disease and infection can occur by few numbers of oocysts. Signs are typically characterized by copious watery diarrhea with weight loss as well as abdominal discomfort, dehydration is very dangerous sign may cause mortalities, fatigue, cramp, vomiting. In addition, asymptomatic infection can occur according to immune state and age of animals. As an emergent pathogen, it developed many virulence factors to escape from the host immune response and resist many antiprotozoal compounds, which in turn is responsible for huge economic losses in the form of calf mortalities, decrease milk productions, meat production. In the current study, we discussed animals and humans’ clinical implications, virulence factors, host pathogen interactions, recent trends in prevention and control and recent developed drugs and treatment. Finally, this comprehensive review has presented an updated view of the status and future perspectives in the field of cryptosporidiosis, which will help in controlling it worldwide.

Keywords: Cattle; Cryptosporidia; Economic; Infectious.

INTRODUCTION
Cryptosporidiosis as an important zoonotic disease infecting humans and animals is becoming increasingly popular in recent years (Pumipuntu and Piratae, 2018). Cryptosporidium is an internal protozoan that is a foremost reason for worldwide diarrhea in both animals and people and infection in animals may result in higher agricultural costs and output losses (Gerace et al., 2019). Cryptosporidium spices including both the serovar C. parvum and C. hominis are a chief cause of diarrhea that could affect young children all over the world. While humans infected only by the serovar C. hominis as well as C. parvum contribute to zoonosis (Leitch and He, 2011). Cryptosporidium is transmitted through the fecal-oral route by the consumption of oocysts from polluted materials such as food or water and through connections between animals. Infectious oocysts that have thick walls resist disinfection such as chlorination, so it is making difficulty in the elimination process from swimming pools, animal housing services, and even the environment (Pinto and Vinayak, 2021).
Cryptosporidium species are protozoa with apicomplexa that involved sexual and asexual life cycle reproduction that can occur in one host. Although the presence of many Cryptosporidium species in cattle but only C. parvum is linked with clinical illness in newborn calves. Transmission by oral-fecal route (Thomson et al., 2017). The investigation of the complete genome sequencing analysis of Cryptosporidium may help in understanding and development of the actual drug, however, the in vitro and in vivo studies are not completely consistent (Rahman et al., 2022). In our review study, we put spotlight on cryptosporidiosis importance as protozoal disease cause enteric disturbance. Whenever, calf diarrhea due to cryptosporidiosis is important as leads to severe economic loss due to delayed body gain in calves and mortalities. Therefore, clinical diagnosis, laboratory diagnosis as serology and PCR were mentioned in this study. The treatment and control of such diseases were also declared, and our conclusion and recommendations were included.

1- History of the Cryptosporidium agent
After two years, he detected another species, C. parvum when checked house mice varies from C. muris in the morphology (oocysts were smaller) beside its fondness site in the small intestine particularly in epithelial layer.
In 1955 a discover other species, C. meleagridis, definitely causing diarrhea and deaths in turkey chicks (Akiyoshi et al., 2003), only in 1980s Cryptosporidium really entered veterinary medicine with reports associated with Cryptosporidium diarrhea in calves (Robertson et al., 2014). in the 1990s inaugurated that Cryptosporidium is the chief enteric-protozoa causing diarrhea in neonatal calves. the first two case informed for cryptosporidiosis in human were announced In 1976 (Hunter and Nichols, 2002). In1982, Ernest Edward Tyzzer (1875-1965) identified the genus Cryptosporidium (C.). Tyzzer discovered the protozoan from the domestic mice stomach glands and defined its different developmental phases including (sporozoites, schizonts, macrogamont, microgametes, macrogamont, and oocyst) as well as its fastidiousness as an "attachment organ" in 1907. Tyzzer then discussed the likelihood of a monoxious development phase and the faecal-oral cycle. He predicted the creation of a genus Cryptosporidium (C.) that mean (crypticus, latin for hidden; here concealed sporocyst) with the specific species C. muris, as well as probable auto-infection and extracellular growth stages, which were validated electron microscopically(Leitch and He, 2011). After two years, he detected another species, C. parvum when checked house mice varies from C. murisin the morphology (oocysts were smaller) beside its fondness site in the small intestine particularly in epithelial layer.
In 1955 a discover other species, C. meleagridis, definitely causing diarrhea and deaths in turkey chicks (Akiyoshi et al., 2003). In1982, Cryptosporidium was reported by “Center for Disease Control” in US on Cryptosporidium related diarrhea associated with immune deficient persons, Milwaukee, Wisconsin, USA were disturbed by C. hominis as a result of contaminated drinking water consumption, to be a waterborne outbreak (Zahedi et al., 2016).

2-Cryptosporidiosis in Egypt
Because of the sampling plan, form of populations considered, management, location, season, and hygienic conditions as shown in table (1) (Ghenghesh et al., 2018) there is a significant variation in Cryptosporidium species prevalence among different countries and in many geographical
locations within a country. In Egypt, for example, unreliable if not inconsistent search results for prevalence several Egyptian organizations initiated investigation in livestock animals and also humans aiming Cryptosporidium spp. (Mahfouz et al., 2014), (Amer et al., 2010), (Amer et al., 2013), (Naguib et al., 2018), (El-Khodery and Osman, 2008), (Abdelaziz et al., 2022), (Elmahallawy et al., 2022), (Ahmed Helmy Abdelsamad Mohamed Tierärztin aus Alexandria and Berlin, 2014), (Helmy et al., 2014), (Bessat M N et al., 2019), (Abd-El-Wahed, 1999), (Aboelsoued et al., 2020), (Ibrahim et al., 2016), (Shaapan et al., 2011). By the usage of various methods of diagnosis built on microscopical, serological and molecular techniques or mixtures of these methods.

Table (1): Cryptosporidiosis in Egypt.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Prevalence</th>
<th>Diagnosis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kafr El Sheikh</td>
<td>Buffaloes: 1.29%</td>
<td>Microscopy examination</td>
<td>(Mahfouz et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>Young Cows: 4.17%</td>
<td>DNA subtyping sequencing and phylogenetic analysis</td>
<td></td>
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<td></td>
<td>Adults: 0.48%</td>
<td>Analysis, RFLP-PCR</td>
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<td></td>
<td>Cattle: 7.07%</td>
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<td></td>
<td>Heifers: 10.20%</td>
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<td></td>
<td>Sheep: 2.50%</td>
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<tr>
<td></td>
<td>Lambs: 4.40%</td>
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<tr>
<td>Kafr El Sheikh</td>
<td>30.2% in calves</td>
<td>Microscopically molecular analysis, sequence analysis</td>
<td>(Amer et al., 2010)</td>
</tr>
<tr>
<td>Dakahlia, El-Gharbia, and Damietta</td>
<td>9.7% in calves</td>
<td>PCR-RFLP analysis, PCR-sequence analysis</td>
<td>(Naguib et al., 2018)</td>
</tr>
<tr>
<td>Behera Menoufiya, Qalyoubiya, Assiut, Sohag</td>
<td>7.59% in calves</td>
<td>Microscopic examination, Conventional PCR, Gene Sequencing and Phylogenetic Analysis</td>
<td>(Abdelaziz et al., 2022)</td>
</tr>
<tr>
<td>Assiut governorate, upper Egypt</td>
<td>38.27% among cattle and 28.16% among buffalo</td>
<td>Parasitological Examination for the Fecal Samples, Nested PCR Procedure</td>
<td>(Elmahallawy et al., 2022)</td>
</tr>
<tr>
<td>Ismailia</td>
<td>Buffaloes: 23.7%</td>
<td>Parasitological Serological Molecular</td>
<td>(Ahmed Helmy Abdelsamad Mohamed Tierärztin aus Alexandria and Berlin, 2014)</td>
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<tr>
<td></td>
<td>Cows: 22.5%</td>
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<td></td>
<td>Sheep: 20.9%</td>
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<tr>
<td></td>
<td>goats: 25.9%</td>
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<td></td>
<td>Dogs: 2.6%</td>
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<td></td>
<td>Wild rats: 6.3%</td>
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<tr>
<td>Region</td>
<td>Prevalence and Methods</td>
<td>Year</td>
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<tr>
<td>Ismailia</td>
<td>In herds: 73.3%. Individual cases in the herd: 32.2%. Prevalence not affected in between cattle and buffaloes</td>
<td>2014</td>
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<td>Parasitological diagnosis Serological diagnosis, Molecular diagnosis</td>
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<tr>
<td>Behera</td>
<td>Calves: 43.2%, Human: 16.1%, Chicken: 6%</td>
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<td></td>
<td>Microscopical examination</td>
<td>Bessat M N et al., 2019</td>
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<tr>
<td>Dakahlia and Kafr El Sheikh</td>
<td>14.2% in buffaloes</td>
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<td></td>
<td>Macroscopically</td>
<td>El-Khodery and Osman, 2008</td>
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<tr>
<td>Qalubia</td>
<td>Modified Zeihl-Neelsen stain: 68.3%, Safranin-methylene blue: 48.3%, Giemsa stain: 30% in lambs</td>
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<tr>
<td></td>
<td>Microscopical examination</td>
<td>Abd-El-Wahed, 1999</td>
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<tr>
<td>Qalyoubiya</td>
<td>40% in buffalo-calves</td>
<td></td>
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<td></td>
<td>Microscopic examination ELISA Cytokines analysis</td>
<td>Aboelsoued et al., 2020</td>
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<tr>
<td>Nile River Delta provinces</td>
<td>13.6% in dairy cattle</td>
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<td></td>
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<tr>
<td></td>
<td>Microscopy, PCR-RFLP analysis and DNA sequencing</td>
<td>Amer et al., 2013</td>
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<tr>
<td>Beni-Suef</td>
<td>Cattle: 10.2%, Buffaloes: 12.3%, Humans: 19%</td>
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<td></td>
<td>Microscopic examination molecular RFLP analysis for COWP Sequence analysis</td>
<td>Ibrahim et al., 2016</td>
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<td>Giza</td>
<td>Native Quails: 31.9%</td>
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<td>Fecal examinations Serological assay Modified Agglutination Test Latex Agglutination Test</td>
<td>Shaapan et al., 2011</td>
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<tr>
<td>Menoufiya Sadat City area</td>
<td>64.1% in newly born calves till 2 months</td>
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<td></td>
<td>Microscopic examination, Conventional PCR, Gene Sequencing and Phylogenetic Analysis</td>
<td>To be published</td>
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3-Clinical Implications

3.1 Disease in calves

Diarrhea, yellowish mucous membranes, anemia, dehydration, and emaciation are the most observed clinical symptoms in animals infected by Cryptosporidium (Elmahallawy et al., 2022). C. parvum is an important cause of diarrhea and also the enteric disease in the newly born calves, which can have notable strong influence on the animal health and economics reverberation for agriculturalists (Shaw et al., 2020). According to (Åberg et al., 2019) there are main four species of Cryptosporidium are found in cattle and responsible for diarrhea including C. bovis, C. andersoni, C. parvum, and C. ryanae. Additionally, C. parvum is the only species that infects the intestines in cattle and humans. Unlike C. parvum, C. bovis has not been attendant with post-weaned calves’ diarrhea. Meanwhile, C. andersoni infects the juvenile abomasum, and adult animals (Åberg et al., 2019). Cryptosporidiosis could cause watery non-bloody diarrhea it may be profusely and prolonged. Other signs including nausea and vomiting. Occasionally, malaise, anorexia, anorexia and weakness (Bouzid et al., 2013) Cryptosporidiosis characteristic diarrhea and abdominal pain could be usually the symptoms of attention, so that a laboratory diagnosis to cryptosporidiosis done. The severity and persistence of disease are characteristically depend on the variability of protozoal characters and the host factors such as the immune status and exposure to the infected one. The severity of the disease caused by Cryptosporidium can ranged from an asymptomatic signs with dissemination of oocysts to aviolent disease although self-limiting illness can occur in case of immunocompetency (Sponseller et al., 2014).

3.2 Human Infection

Cryptosporidium is known as zoonotic protozoan of public concern for human and animals and is considered the second identified etiology of diarrhea and high deaths in young childrens (Khan et al., 2018). There are many Cryptosporidium species identified such as C. meleagridis, C. cuniculus, C. felis, and C. canis in humans nonetheless, both C. parvum and C. hominis responsible for almost more 90% of human cases(Ryan et al., 2021).The most human’s cases reported with immunocompetent individuals. If the host is an efficient antiparasite immune response, recovery from transit diarrhea occur after 2 weeks without treatment. While in immunocompromised individuals intense diarrhea usually continue (U. Ryan et al., 2016), which can be fatal. Certad et al. have isolated C. parvumI12A15G2R1 subtype from the patient’s stools. Then, they inoculated it into SCID mice, which induced invasive gastrointestinal and biliary adenocarcinoma Immune competent individuals’ occurrence a short-term self-limiting disease can occur in case of age up to 2 to 3 weeks. In immune compromised patients, illness can occur with continual symptoms result by dehydration and worsening and may cause mortalities. Additionally, immunocompromised patients can demonstrate unusual signs, such as biliary, respiratory tract disease, uncharacteristic gastrointestinal symptoms, and pancreatitis.

In case of oocysts inhalation in immunocompromised patients, which was supported experimentally by intranasal infections of piglets and laryngotracheitis with mild diarrhea were main observed signs. Although, the almost knowledge about Cryptosporidium transmission suggested the waterborne transmission but it is constitute only a small percentage of
cases as well as the pattern of the transmission routes for many endemic diseases may not identical (Bouzid et al., 2013). Up till now, about 31 Cryptosporidium species have been known, and nearly about 7–12 Human cryptosporidial species are isolated including C. parvum and C. hominis that manifested clinically by symptoms such as abdominal discomfort, fever, vomiting, malabsorption, and profuse diarrhea. The host immune response has a key influence on the disease severity and prognosis. Thus, means that the immunocompetent individuals exhibited self-limiting diarrhea with transitory gastroenteritis and rapid recovery usually occur without treatment within 2 weeks. In the other side, in immunocompromised patients, as HIV/AIDS patients frequently may suffer from inflexible fatal diarrhea. In three years a survey carried out on 22000 children’s at 7 sites in Africa and Asia performed by Global Enteric Multicenter Study to detect the causes of diarrhea and they concluded that Cryptosporidium was the second cause of severe diarrhea after rotavirus infection (U Ryan et al., 2016).

4-Virulence Factors

It is though that about 25 supposed virulence elements documented in C. parvum and C. hominis genome that reflect the host-pathogen interactions which mainly related to the adhesion, locomotion, invasion and amplification of host factors that share in the infection possibilities (Bouzid et al., 2013). Specific virulence factors for Cryptosporidium not discriminated for clearing their way to cause harm to the host or showing that their deactivation consequences in a reduction to the disease solemnity. In vitro cultivation persists difficult to employ contrasting other related parasites species such as Toxoplasma and Plasmodium, and the genetic techniques could be changed in the association with these parasites. Cryptosporidium virulence factors include genes that involved in the processes of host cells parasite interaction, involving connection, attack, excystation, motility, maintenance inside the host, and damage host cells. The virulence factors are markedly differ within interspecies and inter isolates virulence have been documented (Bouzid et al., 2013). The identified factors associated with parasite until the day important in studying the host-parasite relationship. These involve the thomboespondin associated adhesion proteins (TRAP-C1) P23, CP47, Cpa135, CPS-500, circum sporozoite like protein (CSL), GP900, GP60 (proteolytic ally cleaved into GP40/15 mature glycopeptides, mucins (CpMuc4, CpMuc5) and mucin like glycoproteins and C-type lectin (CpClec). Also during gliding locomotion of sporozoites the majority of them are shed according to their attachment within the host cell, localization or reticence of infection by antibodies, these proteins elements seem to play a significant effect in the host invasion and attachment. Up until now, no complete recognition about the actual role of these secreted proteins and their interaction role during the attachment and invasion process, of the parasite as well as the molecular mechanism associated with these interactions. The existence of CRISPR/Cas9-mediated genetic affair of thought to be permit the anticipation of genes encoding for TK and dihydrofolate reductase-thymidylatesynthase (DHFR-TS) and revealed that the role of TK through their contrasting route to pyrimidine nucleotide production in the nonappearance of DHFR-TS. Also, many enzymes in the single purine nucleotide uniting step may be genetically wasted with no influence on protozoal growth like
IMPDH, GMPS, adenosine kinase, and the adenosine transporter thus telling that the protozoa acquire the purine nucleotides coming from host cell (Pinto and Vinayak, 2021).

5- Host-Pathogen Interaction
Derek J. Pinto et al. have discussed their present acquaintance of host-parasite interactions. It is too important to comprehend these interactions to recognize the key biological mechanisms for the discovery of the effective vaccines could be used and drugs that can help to red out of cryptosporidium. It is very important to understand the relationship between Cryptosporidium and host to discover new drugs and vaccines. There are many advanced technology such as the molecular genetics development to operate the protozoal genome to discover new parasitestructures and confirmation of drug targets, fortunate in vitro for protozoa reproduction (Pinto and Vinayak, 2021).

5-1-1 Innate immune response
The initial line of defense against C. parvum infection is the intestine's native immunity, which includes its gut epithelium and distinct innate immune cells. The adaptive immune response begins by innate immunity, which hinders protozoa replication. So, in order establish health attenuation approaches that minimize Cryptosporidium's implications on our health, agriculture, and surroundings, we need to have knowledge of the local host's innate immunity response to C. parvum infection (Crawford and Kol, 2021a).

The intestinal lining epithelial cells establish a natural barrier between the lumine content and internal tissues. Because C. parvum only infects the intestinal epithelium which is the most significant line in the immune response to C. parvum. Intestinal infection with C. parvum led to activation of the inflammatory transcription factor NF-kB and increased expression of the long noncoding RNA NR_045064 and transcription of inflammatory mediators. Furthermore, Toll-like receptor-2 (TLR2) and TLR4 mediate the response of CXCL8 (aka IL-8) and TNFa. The activation of TLR2 and TLR4 by C. parvum infection and NF-kB nuclear translocation leads to the release of LL-37 and b-defensin-2 as antimicrobial peptides (Kumar et al., 2018). C. parvum infection through the NOD-like receptors (NLR) activate the inflammasome complex as a result to that IL-18 produced and elevated in human epithelium and that is considered an significant innate response to infection (Crawford and Kol, 2021a). IL-1b, another inflammatory product that activated, was not augmented post-
infection, and has no influence on infection defenselessness in IL-1b mice. Protozoal shed was markedly enhanced in mice deficient NLRP6, that promotes IL-18 production, however, not in mice missing other NLRs that create inflammatory substances. In contrast to other pathogens, the tiny positive-charged polypeptides exhibit antibacterial characteristics. Phospholipases and antimicrobial peptides such as b defensing 1, b-defensin-2, and LL-37 may kill *C. parvum*. As a result, chemokine and cytokine production from infected epithelium provides a crucial pathway for specific immune cells to clear parasites (Crawford and Kol, 2021a). Mice become more susceptible to infection in case of chemokine receptors deficiency. Trophozoite that formed after infection inhibits apoptosis, so facilitate the growth of the protozoa in the host cell, through inducting the anti-apoptotic factors production as BCL-2, survivin, and osteoprotegerin. As both sporozoite and merozoite phases of protozon are emerged, the inhibition is lost and apoptosis undergoes by host cell (Mead, 2023).

### 5-1-2 Adaptive immune responses

The infection of Cryptosporidium is restricted to CD4+ T cells; the function and relevance of CD8+ T cells is less understood. Previous research showed that CD8+ T cell tracks increased following Cryptosporidium infection and CD4+ and CD8+ T cells gathered from individuals who were earlier infected with Cryptosporidium may produce IFN- in response to infection with *C. hominis* antigens. Likewise, in vitro experiments revealed that CD8+ T lymphocytes donated by donors who had previously been exposed to Cryptosporidium were capable of lysing *C. parvum*-infected intestinal epithelial cells via the release of cytotoxic granules. The role of humoral immunity during Cryptosporidium infection is not actually unknown. The passive vaccination studies in animal models have revealed a link between anti-Cryptosporidium antibody treatment and decreased oocyst shedding and infection (Ludington and Ward, 2015).

<table>
<thead>
<tr>
<th>Immune Response</th>
<th>Functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Innate immune response</strong></td>
<td>Intestinal epithelium</td>
<td>Physical barrier between internal tissue and luminal content</td>
</tr>
<tr>
<td>IFNs</td>
<td>IFN-Y mediate gut epithelium defense against non-viral pathogens.</td>
<td></td>
</tr>
<tr>
<td>Spealized immune cells</td>
<td>NK cells</td>
<td>IFN-Y production &amp; cytolysis of infected cell</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>• Secrete cytokines IL-6, IL-1B, IL-12, IL-18, TNFα, type 1 INFs</td>
<td></td>
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<tr>
<td></td>
<td>• Imprisonment <em>C. parvum</em></td>
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</table>
antigen in the mucosa of gut then travel to drain in the lymph nodes

Macrophage cells  2nd source of INF-Y, phagocytosis
Neutrophils  Infiltrate intestinal mucosa during infection

Adaptive immune response  cellular immune  Cytotoxic T-cells ex: CD+ T-cells, TRAIL
Humoral immune response  Serum or fecal B-cells  Decrease level of IgA, IgE, IgG
Normal or increase level of IgM (Pantenburg et al., 2008) (Chattopadhyay and Mahapatra, 2019a)

6-Diagnosis

6-1 Macroscopic Examination of the Fecal Samples
The fecal samples were inspected macroscopically to detect irregularities in consistency and color, the presence or absence of blood, the state of digestion, and the presence of mucus or other unusual ingredients (Elmahallawy et al., 2022).

6-2 Antigen detection
6-2-1 Microscopic examination
Microscopic Detection of Cryptosporidium in laboratories is via stains and/or fluorescent antibodies (IFA). Although microscopy is relatively simple instruments and cheap edible, but it need labour rigorous, requires operator experts and lacks sensitivity and specificity. Due to oocysts similarity in the size and also the shape of yeasts fecal parts and other things debruised, differential staining techniques are usually required, modified Ziehl–Neelsen staining is used as a differential staining technique (U. Ryan et al., 2016).

6-2-2 PCR analysis
PCR for identifying Cryptosporidium is more sensitive compared to standard microscopy and serological approaches, while help in species and sub-species identification of these organisms. The introduction of real-time PCR provides a viable alternate to traditional approaches. Multiplex PCR has become an accepted method for determining the presence of numerous intestinal parasites in a single reaction. In preliminary experiments, multiplex qPCR approach were found to be 100% sensitive and specific when compared to uniplex qPCR method. Yang et al. used dPCR quantitative technique for Cryptosporidium oocysts. The advent and rising ubiquity of next generation sequencing (NGS) technology is prospective to impact the biology of protozoan criteria during the next decade. Nested PCR was also used (O’Leary et al., 2021).

6-3 Serological examination
The detection of Cryptosporidium speciesby immunofluorescence required the presence of a fluorescent microscope, which has limited the application of this technique, the quantitative enzyme immunoassays (EIA) and enzyme linked immunosorbent assays (ELISA) overcome these disadvantages. The diagnostic usage of EIA and ELISA kits allowed more sensitivity and specificity with nearly (94 and 100%) respectively in compared with acid-fast staining methods.
However, enzyme-based immunological detection of Cryptosporidium may be low sensitivity in case of few oocysts in the tested samples (O’Leary et al., 2021).

7-Recent Trends in the Prevention and Control of Cryptosporidiosis

7-1 Treatment

There are many aims have been accepted for chemotherapy and advances has been made on drugs for these goals that cover the parasite vital processes such as dynamics, nucleic acid production, proteases, and lipid metabolism. Other groups also have carried out to identify prospective drugs. Drugs advanced for treatment of cryptosporidiosis has been hurt by a limited of success. Also, the included many obstacles between in vitro and in vitro efficacy of the applicable drugs activists. To maintain a assorted development pipeline, the research should be continue to realize the success for effective drugs for Cryptosporidium infection (Wang et al., 2020). Nitazoxanide is used in the adult, immunocompetent patients, but is not effect in the other susceptible populations as infants (Crawford and Kol, 2021b). Nitazoxanide is the main available and approved drug by FDA in malnourished children and immunocompromised patients with questionable efficacy. Although, Triacsin C as a drug candidate, which aims the parasite’s long-chain fatty acyl coenzyme A synthetase enzyme (LC-FACS), a serious component of the fatty acid metabolism pathway that may be used (Chattopadhyay and Mahapatra, 2019b). New researches target the molecule R134 proposed on its capability to hits the C. parvum LC-FACS enzyme isoforms as well as some chemical properties such as its high binding affinity, stability and reasonable absorption, distribution, metabolism, excretion and toxicity properties comparable to those of the Triacsin C (Chattopadhyay and Mahapatra, 2019b). A parasite cysteine protease inhibitor was also effective in vitro and in an animal model (U. Ryan et al., 2016). Moreover, clofazimine drug is used in treatment of Cryptosporidium infection although, its concentration was found to be lower that the effective dose in clinical studies. Therefore, the clofazimine will provide a therapy for cryptosporidiosis patients currently without safety and effective treatment with possibility of improvement of oral absorption are developed in the future (Zhang et al., 2022).

7-2 Vaccination

Concerning the mode of action of the tested vaccine, difference was observed according to the postulate used, with 30% hold back parasite entry into cells, 10% break up the biological cycle of the parasite, in 30% the arousal of protective immunity in the host and in 10% the performance verified the immunogen in the process related to parasitic adhesion and invasion to host cells. In the other artefacts, the style of action of the tested vaccines was not mentioned. In the selected studies there were no affairs related to the costs of vaccines in tests. In all data banks, the same search countenance was inserted, which refine the results, so the application of riddle makes the methodology more sensible, they are: Cryptosporidium spp. AND vaccine; Cryptosporidium spp. AND protein vaccine; Cryptosporidium spp. AND animal vaccine; Cryptosporidium spp. AND DNA vaccine; Cryptosporidium spp. AND vector vaccine. To bring out the data that refute the question of this RSL, fields were created that organized the information that should be noticed, such as get going the study population; principle, modes of action of the tested vaccine, as
well as its efficacy and cost benefit (Silva et al., 2021).

8- Conclusion

Cryptosporidium infection is a major problem, especially in calves. C. parvum is the major strain found in recent parturiated calves. C. parvum causes neonatal diarrhea syndrome in calves. Economic losses are mortality, morbidity, decreased in productivity, increased veterinary costs for drugs and increased labour. Diagnosis of such disease depends mainly on oocyst detection in feces as a gold standard test, for accurate diagnosis PCR application is recommended. Prospective studies on genome of cryptosporidiosis is needed. As, no efficient effective drug available for cryptosporidiosis treatment, application of effective hygienic measures should apply, sanitation and of the premises is needed to prevent spread of such disease.

BIBLIOGRAPHY


Amer, S., Zidan, S., Adamu, H., Ye, J., Roellig, D., Xiao, L., Feng, Y., 2013. Prevalence and characterization of Cryptosporidium spp. in dairy


Mahfouz, M.E. Isaye., Mira, N., Amer, S.,


in Farmed Animals. Cryptosporidium parasite Dis. 149. https://doi.org/10.1007/978-3-7091-1562-6_4


https://doi.org/10.33448/RSD-V10I6.15540


