UPDATE IN DIAGNOSIS AND TREATMENT OF CRYPTOSPORIDIOSIS AN UPDATE REVIEW
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Abstract

Cryptosporidiosis represents a major public health problem which transmitted by contamination of food or water by sporulated *Cryptosporidial* oocyst. Causing self- limited diarrhea in immunocompetent person and chronic and life threatening diarrhea among immunocompromised individuals. It can be diagnosed by concentration and detection of its Oocyst in different environmental samples and water by microscopic and immunological examination such as enzyme immunoassay (ELISA) for parasite antigens and nucleic acid amplification assay as well as use of molecular techniques such as Polymerase Chain Reaction (PCR).

Treatment is face challenges, Macrolides, Paramomycin, Nitazoxanide and Mirazid. All these drugs have partial efficacy in reducing disease severity in immunocompetent individuals. Nitazoxanide has partial efficacy in immunocompromised individuals. Resolution of Cryptosporidiosis can be maintained with effective Highly Active Antiretroviral Therapy (HAART).

Keywords: Cryptosporidosis, *C. parvum*, *C. hominis*, diarrhea, diagnosis, Treatment.

Introduction

Cryptosporidium is a globally distributed genus of protozoan parasites infecting the gastrointestinal tract of a wide range of invertebrate and vertebrate hosts, including humans (Xiao and Feng, 2008). The majority of human infections are caused by two species; the anthroponotic *Cryptosporidium hominis* and the zoonotic *C. parvum* (Waldron *et al*, 2010). Cryptosporidiosis causes chronic and life threatening diarrhea among immunocompromised individuals but self-limiting diarrhea in immunocompetent individuals (Banwat *et al*, 2004).

Cryptosporidiosis is normally initiated by ingestion of *Cryptosporidium* oocytes from contaminated drinking water, recreational water, *Cryptosporidium* is recognized as a major human waterborne pathogen worldwide (Clancy *et al*, 2007). *Cryptosporidium* is recognized as a public health issue world-wide (Yoder and Beach, 2010) whereas; *Cryptosporidium* sp. has been estimated at 60,000 cases per year (Bridge *et al*, 2010). Patients with Acquired Immunodeficiency Syndrome (AIDS) may be susceptible to a wide range of *Cryptosporidium* sp. and genotypes to which an immunocompetent individual is not susceptible (Plutzer and Karanis, 2009). Diagnosis of cryptosporidiosis has progressed from histological identification in intestinal biopsies to microscopic examination of fecal specimens for infective oocysts, enzyme immunoassay for parasite antigens and nucleic acid amplification assay as well as use of molecular techniques such as Polymerase Chain Reaction (Phillip *et al*, 2008).

Taxonomy and Biology: *Cryptosporidium* is classified within the coccidian parasites; some studies have revealed that the genus belongs to an early emerging taxonomy of the Apicomplexa, and that it is more closely related to the *Gregarinia* than to the *Coccidia* (Rosales *et al*, 2005). The genus *Cryptosporidium* is one of over 300 genera that include 4,800 named species in the phylum Apicomplexa (Fayer and Xiao, 2008). It comprises several species and genotypes which infect a

wide range of host including humans. There is extensive genetic variation within the genus *Cryptosporidium*, with at least 23 species being considered valid by most investigators, these species are collectively found in human, mouse, cattle, pig, sheep, horse, goat, cat, dog, kangaroo, chicken, turkey, fish, ferrets, lizard, monkey and deer (Smith, 2008). With a few exceptions, most species and genotypes are host-adapted in nature, of these 23 species, at least 7 have been found to infect human immunodeficiency virus (HIV)-infected individuals i.e. *C. parvum, C. hominis, C. meleagridis, C. felis, C. muris, C. canis and C. suis* (Traversa, 2010). Abd El Kader *et al.* (2012) in Egypt by genotyping stated that *Cryptosporidium hominis* and *C. parvum* are the main agents of cryptosporidiosis in humans. They examined a total of 391 human faecal samples were collected, between May 2008 and March 2009, from ten public hospitals in Great Cairo. Initial screening by immunochromatographic detection kit "the Stick *Crypto-Giardia*; Operon" showed 23 possible positive cases. Twenty of them were confirmed by microscopic examination. PCR was performed by amplification of the oocyst wall protein (COWP) gene where 18 out of 23 samples were positive, one not detected by microscopy. *Cryptosporidium* genotyping was performed by RFLP analysis of PCR products of the diagnosis PCR. The genotyping distribution was nine cases showing *C. hominis* genotype, three showing *C. parvum* genotype and three showing mixed infection by C. hominis and *C. parvum*. They concluded that the high elevated prevalence of *C. hominis* (80%), most anthroponotic species, suggesting a human-human transmission.

Mode of Transmission: *Cryptosporidium* seems to be a greater public health threat because of its insensitivity to disinfection regimens used in water purification leading to an increased likelihood of infectious oocysts being present in conventionally treated drinking water (Smith *et al*, 2006). The infection is spread to human in a number of ways: from person to person, from animals, via food, and water. Contact with other cases, foreign travel, and contact with livestock are recognized risk factors for human cryptosporidiosis (Hunter and Thompson, 2005), also sexual contact has been reported (Ghimire *et al*, 2004). Airborne transmission has also been postulated (Chalmers and Davies, 2010). There are fewer foodborne outbreaks documented than waterborne outbreaks (Smith *et al*, 2007). *Cryptosporidium sp*. can be transmitted from person to person and zoonotically, both directly and indirectly, through the environment (Siwilia *et al*, 2007). Filth flies transport infectious oocysts of *C. parvum* on their external surfaces and in their digestive tracts deposit infectious oocysts on visited foodstuff (Getachew *et al*, 2007).

Epidemiologic pictures: The broad range in prevalence of cryptosporidiosis may be attributed to many causes as immune state of the patients, patient's age, environmental habitats or seasonal variation (Derouin *et al*, 2010). Human cryptosporidiosis was also reported in different ages whereas; prevalence of *Cryptosporidium* infection in Egyptian patients varied significantly from 0% to 47%, it is a common cause of severe diarrhea among the Egyptian children (Youssef *et al*, 2008) as zoonosis from farm animals (Amer and Harfoush, 2010). The risk of infection may be high and the disease probably exerts most of its impact on neonates and infants (Hunter *et al*, 2009). It has been the cause of multiple diarrhea outbreaks in developed and developing countries (Ng *et al*, 2010). The human immunodeficiency virus (HIV), and the acquired immunodeficiency syndrome (AIDS) patients have been reported to have high prevalence of cryptosporidiosis

(Zaidah *et al*, 2008). In most countries studied, *C. parvum* and *C. hominis* are responsible for greater than 90% of human cases of cryptosporidiosis, with the balance attributable to *C. meleagridis*, *C. canis* and *C. felis* (Xiao and Fayer, 2008).

On the other hand, Sponseller *et al.* (2014) reported that *Cryptosporidium* oocysts may occur by inhalation of aerosolized droplets or by contact with fomites contaminated by coughing. They concluded that delineating the role of the respiratory tract in disease transmission may provide necessary evidence to establish further guidelines for prevention of cryptosporidiosis.

Life cycle and Morphology: *Cryptosporidium* has a complex life cycle including both sexual and asexual reproductive stages (Thompson *et al*, 2005). All species of *Cryptosporidium* undergo endogenous development, resulting in the production of an encysted stage discharged in the faeces of their host (Fayer *et al*, 2000). After being ingested, the oocysts excyst in the small intestine, they release sporozoites that attach to the microvilli of the epithelial cells of the small intestine. From there they become trophozoites (Borowski *et al*, 2008) that reproduce asexually by the multiple fission the process known as schizogony. The trophozoites develop into Type 1meront that contain 8 daughter cells, which get released by the meronts, can cause autoinfection by attaching to epithelial cells. Others of these merozoites become Type II meronts, which contain 4 Type II merozoites, these merozoites get released and they attach to the epithelial cells. From there they become either macrogamonts or microgamonts (Chen *et al*, 2003). These are the female and male sexual forms, respectively. This stage, when sexual forms arise, is called gametogony, the microgamont penetrating the macrogamonts producing zygotes which develop into oocysts, 20% of oocysts have thin walls and

so can reinfect the host by rupturing and releasing sporozoites that start the process over again. The thick-walled oocysts are excreted into the environment. The oocyst is mature and infective upon being excreted. They can survive in the environment for months (Fayer, 2008).

Pathogenesis and immunology: The mechanism by which *Cryptosporidium* infection causes diarrhea remains unclear, whatever the mechanisms by which *Cryptosporidium* infection causes disease may be, attachment to and invasion of host cells are crucial primary events in pathogenesis (Tzipori and Ward, 2002). The parasite does elicit a local inflammatory response, and increased production of prostaglandins and several cytokines, particularly The Interferons as IFN-Y, has been described, where these inflammatory mediators may consequently alter solute transportation in the intestinal epithelial cell, leading to osmotic diarrhea (Singh *et al*, 2005). Both innate and adaptive immunity are involved in the resolution of cryptosporidiosis and resistance to infection (Thompson *et al*, 2005). The invasion of intestinal and biliary epithelial cells by *C. parvum* in vitro activates epithelial cells, resulting in the production and secretion of various cytokines and chemokines and anti-microbial peptides (e.g., β-defensins and cathelicidins) that can kill *C. parvum* or inhibit parasite growth (Barakat *et al*, 2009). The alterations in miRNA expression profiles in cultured human biliary epithelial cells following *C. parvum* infection have been characterized importantly; miRNA-mediated post-transcriptional gene regulation may regulate expression of genes critical to epithelial anti-microbial defence (Zhou *et al*, 2009).

Clinical picture: *Cryptosporidium* has become one of the most commonly reported enteric pathogens in immunocompetent and immunocompromised person worldwide (Meinhardt *et al*, 1996).

Clinical features in immunocompetent

people: Cryptosporidiosis is an acute self-limiting gastoenteritis in immunecompetent humans which occurs worldwide, and in all age groups, although children especially those under 2 years old are most frequently and severely affected (Chen *et al*, 2002). *Cryptosporidium* sp. infects intestinal or respiratory epithelial cells resulting in self-limiting diarrhea or pulmonary cryptosporidiosis in immunocompetent persons (Xiao and Feng, 2008).

Clinical features in immunocompromised people: Patients with T-cell immune deficiency are at most risk, including those with haematological malignancies (particularly children), and Human Immunodeficiency virus (HIV) patients with cluster of differentiation (CD4+) lymphocyte counts more than 50/mm^3, the groups of high-risk patients frequently experience chronic or intractable disease, the other symptoms include abdominal cramps, anorexia, nausea, vomiting, fatigue and low-grade fever. In such patients, in addition to typical but severe intestinal disease, atypical and extra-intestinal disease can also develop. Patients can have chronic watery diarrhea that can last more than 2 months and shed oocysts in the stool during the entire period, resulting in severe dehydration, weight loss and malnutrition, extended hospitalizations and mortality (Abubakar *et al*, 2007). Adamu *et al*. (2014) in Ethiopis stated that cryptosporidiosis is an important cause for chronic diarrhea and death in HIV/AIDS patients and that infection was associated with the occurrence of diarrhea and vomiting. Diarrhea was attributable mostly to *C. parvum* subtype family IIa and *C. hominis*, whereas vomiting was largely attributable to *C. hominis* and rare *Cryptosporidium* species. Calf contact was identified as a significant risk factor for infection with Cryptosporidium spp., especially C. parvum subtype family IIa. They concluded that *C. parvum* is a major cause of cryp-

tosporidiosis in HIV- positive patients and zoonotic transmission is important in cryptosporidiosis epidemiology

The AIDS patients with cryptosporidiosis most commonly have acute or sub-acute onset of watery diarrhea, which may be accompanied by nausea, vomiting, and lower abdominal cramping. Severity can range from the asymptomatic to profuse, cholera-like diarrhea. More severe symptoms tend to occur in immune-suppressed patients, whereas transient diarrhea alone was typical in hosts with competent immune systems. Fever was present in approximately one-third of patients and malabsorption was common. The epithelium of the biliary tract and the pancreatic duct could be infected with *Cryptosporidium*, leading to sclerosing cholangitis and to pancreatitis secondary to papillary stenosis, particularly among patients with prolonged disease and low CD4 cell counts (**De Souza** *et al,* 2004). Pulmonary infections also have been reported may be under-recognized (Mercado *et al*, 2007).

Diagnosis of cryptosporidiosis*:* Diagnosis of Cryptosporidiosis has progressed from histological identification in intestinal biopsies to microscopic examination of feacal specimens for infective oocysts, enzyme immunoassay (EIA) for parasite antigens and nucleic acid amplification assay as well as use of molecular techniques such as PCR (Phillip *et al*, 2008). In the majority of modern clinical laboratories in developed countries, the most widely used staining method for the detection of relatively low numbers of *Cryptosporidium* is still immunofluorescence assay (IFA) (Sunnotel *et al,* 2006). It is often necessary to use an oocyst concentration technique such as Immunomagnetic separation (IMS)-recovered oocysts to maintain an acceptable level of assay sensitivity (Miller *et al*, 2006).

Microscopy: Microscopy method involves concentration of the samples and staining to obtain accurate diagnosis

which achieved by detecting the presence of oocysts, the environmentally resistant forms, in stool samples, mucus, and biliary samples or by determining one of the different stages of *Cryptosporidium* in a biopsy of intestinal tissue (Berger, 2006).

Immunological-based detection methods: Application of these genetic tools has led to enhance knowledge and understanding of systematics, biology, epidemiology, ecology and population genetics of *Cryptosporidium* sp., improving the strategies for the prevention, surveillance and control of cryptosporidiosis in humans and other animals (Smith *et al*, 2006).

1- Immunofluorescence assay (IFA): IFA procedures that employ *Cryptosporidium*-specific polyclonal or monoclonal antibodies is developed to aid in the identification of oocysts in stool and environmental samples (Garcia *et al*, 1997). Antibodies specific to *Cryptosporidium* have been detected, using immunofluorescent assay (IFA) procedure, in sera obtained from persons who recovered from confirmed infections, it is important since it can be done in combination with membrane exclusion / permeability dyes to estimate oocysts viability (Campbell and Current, 1998). IFA technique is not required to distinguish between *C. parvum* oocyst and non-*C. parvum* oocysts, it is time consuming, allows only few samples to be examined per day, and the necessity for an expert or experienced personnel to interpret the results that may not be available, (Nair *et al,* 2008).

2- Enzyme immunoassay (antigen detection): These enzyme immunoassays have become widely accepted as methods for screening stool samples to detect *Cryptosporidium* and other parasitic organisms (Pieniazek *et al,* 2002). It is developed to detect the antigens of *Cryptosporidium* species (Shimizu *et al,* 2000). The detection of *Cryptosporidium* antigens in faecal samples (coproantigens) is

another diagnostic approach. Sensitivity of coproantigen detection can be lower than most microscopic approaches (Johnston *et al.,* 2003). Some coproantigen detection assays have advantages over microscopy in that they can detect (prepatent) infections in animals not excreting oocysts in faeces (Smith and Nichols, 2007), and can be employed for the rapid and cost-effective screening of large numbers of faecal samples (Garcia *et al,* 2003). However, like other immunological methods, they do not allow the species or genotype of *Cryptosporidium* involved in the infection to be determined. The expensive nature of the enzyme immunoassay kits used for ELISA places them above the reach of many laboratories in developing countries and this may decrease their routine usage in diagnostic services (Miller *et al,* 2006).

3- PCR based method: Genetic method for detecting *Cryptosporidium* oocysts has recently been developed; the method identifies and amplifies *Cryptosporidium* nucleic acid using PCR (Abe *et al,* 2002). With Integration of flow cytometry, dot blot and magnetic antibody methods could improve the sensitivity of the PCR assay **(**Dixon *et al,* 2002), PCR is sensitive and has the potential for accurate diagnosis in patients who do not presently know the cause of their diarrhea (Oyibo *et al,* 2011), this could have considerable advantage in the treatment of immunosuppressed individuals, allowing for early diagnosis before the onset of symptoms.

A nested PCR assay was developed for detection of *C. parvum* oocysts directly from stool specimens by using a nested primer, this test can detect few oocysts ranging from asymptomatic infections to monitoring response to therapy for clinical as well as the environmental samples (Semenza and Nicholas, 2007). PCR followed by restriction fragment length polymorphism (RFLP) analysis or sequencing has been widely employed. PCR-

based techniques employing specific primer pairs for the selective amplification of different genetic loci, followed by enzymatic cleavage or sequencing, have been used to characterize and classify *Cryptosporidium* species or "genotypes" (Xiao *et al*, 2004). Some key markers (loci) provide a useful genetic marker for the specific identification of *Cryptosporidium,* having relatively low intraspecific and relatively high interspecific sequence variation (Jex *et al*, 2007). PCR cannot, however, be used for routine diagnosis of *Cryptosporidium* oocysts due to several challenges. These include its technical complexity and interference of results by inhibitors, its expensive nature, and time-consuming procedures (Gatei *et al,* 2006).

Concentration techniques for detection of oocysts in water: These methods have been designed specifically to concentrate and identify oocysts in water samples and rely typically on filtration for oocyst concentration, oocyst purification by Immuno-magnetic separation and detection using a direct fluorescent antibody method and/or Nomarski differential interference contrast microscopy for confirmation (Medema *et al*, 2006).

a. Flow cytometry method: Flow cytometry is a laser-based instrument that analyzes particles in a liquid suspension on a particle by- particle basis, it can differentiate and physically separate particles based on their size, internal complexity and fluorescence (Oyibo *et al,* 2011). More recently, flow cytometric methods have relied on the immuno-magnetic separation based (IMS) purification of oocysts over filtration/flotation approaches (Hsu *et al*., 2005), thus providing a significant advance in the rapid screening of environmental samples for *Cryptosporidium* and rapid quantification of oocysts.

b. Immunomagnetic separation (IMS): IMS concentrates *Cryptosporidium* oocysts by using a magnetic bead coated with an anti- *Cryptosporidium* antibody

but the efficiency of the IMS method could be decreased in highly turbid water samples so its use is limited (Fontaine and Guillot, 2003).

c. Fluorescence activated cell sorter (FACS): The use of fluorescence microscopy and the direct fluorescent antibody (DFA) assay using a fluorescein isothiocyanate-conjugated anti-*Cryptosporidium* monoclonal antibody (FITC-C-mAb), which recognises surface epitopes on oocysts, has been reported to achieve relatively high specificities (96–100%) and sensitivities (98.5-100%) for the detection of *Cryptosporidium* oocysts in faecal smears and environmental samples Commercially available a fluorescein isothiocyanate-conjugated anti-*Cryptosporidium* monoclonal antibody (FITC-C-mAb), among which IgM is used routinely for detecting and enumerating oocysts or fecal samples (Smith *et al*, 2007).

d. Fluorescent in situ hybridization (FISH): Most FISH assays for *Cryptosporidium* oocysts rely on the hybdridization to RNA (rather than to DNA), targeting a variable region of the small subunit (SSU) of the nuclear ribosomal RNA (Bednarska *et al*, 2007). FISH was not capable of accurately determining viability, where the extent to which such FISH probes are useful for studies of oocyst viability is dependent on the rate at which small subunit (SSU) rRNA decays, and the rate of degradation will vary depending on different environmental conditions i.e., temperature, pH, salinity and/or RNase contamination present (Smith *et al*, 2004).

e. Electrophoretic mutation scanning: The approaches include mutation scanning methods, such as heteroduplex analysis, temperature gradient gel electrophoresis (TGGE), denaturing gradient gel electrophoresis (DGGE) and single strand conformation polymorphism (SSCP) SSCP is a particularly useful approach in that it relies on the principle that the electropho-

retic mobility of a single-stranded deoxyribonucleatide acid (DNA) molecule in a non-denaturing gel is highly dependent on its size and structure (conformation) and offers the capability of detecting single point mutations in amplicons of < 500 bp (Gasser *et al*, 2006). PCR-based SSCP has proven to be a powerful tool for the identification of *Cryptosporidium* species or genotypes, to screen for genetic variability within and among large numbers of samples, and, importantly, to screen for unknown mutations (Jex *et al*, 2007).

Treatment: No drug or drug combination effectively treats Cryptosporidiosis in humans. Clinical trials have examined the efficacy of Macrolides, Paramomycin, and Nitazoxanide in reducing disease severity in immunocompetent individuals (Mor and Tzipori, 2008). Only Nitazoxanide has partial efficacy in immunocompromised individuals (Tandon and Gupta, 2014). Resolution of cryptosporidiosis was maintained with effective highly active antiretroviral therapy; HAART (Zardi *et al,* 2005).

Massoud *et al*. (2008) in Egypt reported that sixty cryptosporidiosis patients from Mansoura University Hospitals, 36 males and 24 females, with the age from few months to ten years (mean age 6.1) were divided into three cross-matched groups of 20 patients each. All patients received the glutamine-based oral rehydration solution with 111 mmol/l glutamine, 20 mg zinc acetate once a day and vitamin A supplementation (200,000 IU) once a day for 2 weeks. For cryptosporidiosis treatment, G1 received Mirazid (10 mg/kg for 2 weeks), G2 received Paromomycin (500 mg qid for 2 weeks), and G3 received a combination of Mirazid (10 mg/kg) and Paromomycin (500 mg) for two weeks. The result was assessed according to the scales: 0 = no improvement, 1 = symptoms began improvement (reduction of diarrhea frequency and stool volume, less abdominal pain, less nausea & vomiting),

2 = diarrhea eradication, 3 = weight gain, 4 = oocyst counts reduction, 5 = reduction in diarrhea and oocyst counts, 6 = eradication of diarrhea & oocysts. G3 showed significantly higher difference than G1 & G2 in the 1st week (p =.036, 0.025 respectively), no significant difference in 2nd week, a significantly higher difference than in G1 (0.003), & G2 (0.006) in 3rd week, and a significantly higher difference than G1 (0.014), & G2 (0.01) in 4th week, but without significant differences in oocyst shedding in the 3 groups.

Also, Hussien *et al*. (2013) in Egypt reported that ninety children infected with *C. parvum* at Assuit Al-Azhar University Hospitals were chosen (60 males & 30 females) with age range from 6 months to ten years were divided into two groups of 45 patients for each (G1 & G2). All patients suffered from chronic diarrhea for more than fifteen days. Cross-matched 45 children suffering from chronic diarrhea were used as a control group (G3). Stool samples were collected and examined for detection of *Cryptosporidium* oocysts using Sheather's sugar and Modified Ziehl-Nelseen stain techniques. The first group (G1) received Nitazoxanide (100 mg and 200 mg every 12 hours for 3 days for children aged 6 months to 3 years and chil dren aged 4 to 10 years respectively), G2 received Paromomycin (25mg/kg/day for 2 weeks). Third group received placebo. Significant improvement and shortening of the duration of diarrhea occur in G1; of 45 patients received Nitazoxanide 39 cases showed complete clinical and laboratory cure (86.6%), 5 cases showed clinical improvement with reduction in the number of oocysts and 1 case showed no cure. In G2 of 45 cases received Paromomycin 31 cases showed complete cure (68.8%), 8 cases showed clinical improvement with reduction of oocysts number and 6 cases were not cured. Nitazoxanide proved highly effective than Paromomycin in cryptosporidiosis.

1- General measures and medication: The majority of immunocompetent individuals suffer a short (less than 2 weeks) self-limiting course that requires supportive care with rehydration and occasionally antidiarrhoeal medication and ends with spontaneous recovery (Xian-ming *et al*, 2002).

In immunocompromised individuals, as AIDS patients, Cryptosporidiosis resolves slowly or not at all, and frequently causes a particularly severe and permanent form of watery diarrhea coupled with a greatly decreased ability to absorb key nutrients through the intestinal tract, this result in progressively severe dehydration, electrolyte imbalances, malnutrition, wasting, and eventual death (Murray *et al*, 2005). The severity of Cryptosporidiosis depends on the immune state of the host (Woods and Upton, 1998). In HIV-AIDS people's management require antiretroviral and anti-parasitic drugs with fluid and electrolyte therapy (Masur *et al,* 2008).

2-Passive immunotherapy: Passive immunotherapy has been reported to reduce the severity of *Cryptosporidium* infection .Several approaches have been investigated, including the use of bovine colostrums and colostral antibodies (hyperimmune and natural), monoclonal or polyclonal antibodies and lectins specific for certain glycoproteins on the surfaces of sporozoites, merozoites or oocysts, antigens associated with parasite motility, attachment and invasion of the host cell represent opportune targets for passive immunization against *C. parvum* (Riggs *et al,* 2002).

3-Possible use of sporozoite-specific lectins: Lectins are carbohydrate-binding plant proteins which are believed to mediate cell-to-cell interactions, including interactions between host and parasite, Keusch *et al.* (1995) identified a sporozoite-specific lectin adherence factor as the cause of sporozoite attachment to the intestinal surface.

4-Probiotic treatment: Probiotics, also termed biotherapeutic agents, are live microbial supplements which beneficially affect the host by improving its intestinal microbial balance; they have also been shown to limit *C. parvum* infection in immunocompromised individuals in animal models (Alak *et al,* 1999). Probiotics have been used successfully in treatment of acute diarrhoea and also in preventing antibiotic induced diarrhea (D'Souza *et al*, 2002).

5-Treatment of drinking water: The realization that *Cryptosporidium* oocyst are resistant to many chemical disinfectants led to a search for methods that can inactivate oocysts without generating harmful by products (Rochelle *et al,* 2005). Ultraviolet light treatment at relatively low doses will inactivate *Cryptosporidium* this is approved by Water Research Foundation-funded research which discovered UV's efficacy in inactivating *Cryptosporidium* (Murray *et al,* 2005).

6- Vaccination: Mead (2014) stated that vaccine development or immunotherapy that prevents disease or reduces the severity of infection is a relevant option since efficacious drug treatments are lacking. In particular, children in developing countries might benefit the most from a vaccine since cryptosporidiosis in early childhood has been reported to be associated with the subsequent impairment in growth, physical fitness, and intellectual capacity. In this review, immunotherapies that have been used clinically are described as well as experimental vaccines and their evaluation in vivo. Eladl *et al.* (2014) studied the consequence of cryptosporidiosis on the immune response of vaccinated chickens against Newcastle disease and/or avian influenza was studied. They found that H5N2 vaccine at day 10 of chicken life is effective in chickens indicated by the geometric mean of HI titer against AI virus. The findings of this study showed that the infection with Cryptosporidia in the broiler chicken has

a depressive effect on the immune status of the birds vaccinated against ND and/or AI vaccination. Moreover, the obtained protection rates against challenge with virulent ND virus observed to be parallel to the results of HI- test. Also, by using 2 different antigens (one commercial and field prepared antigen) to avian influenza virus, lower Geometric mean (GM) HI titer were appeared in infected and vaccinated group than vaccinated group only. A study of the relative lymphoid organs weight such as bursa of Fabricius from the experimental chicks indicated that those organs were comparable between the groups infected-vaccinated and vaccinated only. Non-significant variations in final live weight between uninfected control and infected groups were indicated. Also, H5N2-AI vaccination at 10 days old did not affect the final live weight. ND and/or AI Vaccination could not be a substitute to application of good hygienic measures and fecal examination of the birds especially for protozoal diseases such as cryptosporidiosis. It could be concluded that cryptosporidiosis could be one cause of ND and/or AI vaccination failure in poultry farms.

Conclusion

Cryptosporidiosis is a real zoonotic worldwide problem particularly among children can be diagnosed by detection of its oocysts in the different environmental specimens using microscopic or immuno-logical or molecular based examination also by using different concentration techniques to water oocysts.

But its treatment face challenges due to the lack of adequate drug or drug combination. Many of drugs have been tested, including Paromomycin, Mirazid with or without Paromomycin, Macrolides (as Azithromycin and Spiramycin) & Albendazole, are partially effective. Nitazoxanide is used to treat cryptosporidiosis in HIV-infected patient and the Antiretroviral therapy (ART) which greatly influences the outcome of cryptosporidiosis.

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