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Review Article
Cryptosporidiosis in Animals and Man: 3. Prevention and Control

[1]Alaa A. Ghazy, [1]Sobhy Abdel-Shafy and [2]Rafaat M. Shaapan

[1]Department of Parasitology and Animal Diseases, Division of Veterinary Research, National Research Centre, Dokki, Giza, Egypt
[2]Department of Zoonotic Diseases, Division of Veterinary Research, National Research Center, Giza, Egypt

Abstract

The control of cryptosporidiosis relies mainly on hygienic measures and good management. Preventive hygienic measures are by far the most effective approach to control this parasite in animals, the objective being to destroy the external forms of the parasite (infective mature oocysts) and to prevent their transmission among animals. The destruction of oocysts on surfaces of housing facilities, pens and parturition buildings is possible using effective disinfectants such as 50% ammonia, 3% hydrogen peroxide or 10% formalin. Measures to reduce transmission between animals should be encouraged. Limiting the number of animals enclosed in the same facilities and avoidance of high stocking rates in the parturition area, maintaining a short calving period, administration of appropriated supplies of colostrum especially hyper immune colostrum from immunized dams, isolation and treatment of diarrheic infected animals; all help to prevent outbreaks of cryptosporidiosis, reduce the spread of infection within a herd and minimize mortality and morbidity in infected herds. Chemotherapeutic agents such as paromomycin, decoquinate, lasalocid, halofuginone lactate, nitazoxanide, dinitrooryzalin, β-cyclodextrin and probioties have proved a potential therapeutic effect against cryptosporidiosis in the form of reduction the duration and numbers of oocysts shedding and the incidence and severity of the diarrhea. Immunization of ruminants during pregnancy with either recombinant *C. parvum* sporozoites surface antigens or plasmid DNA encoding the CP15 or CP23 antigens appear to be a valuable approach for producing colostrum for the passive immunotherapy of cryptosporidiosis. Hyper immune colostrum prevented diarrhea and reduced oocysts shedding in newly born calves, lambs and goat kids exposed to *Cryptosporidium* infections.

Key words: Cryptosporidiosis, oocysts, parturation building, chemotheraputic agents, colostrum *Cryptosporidium parvum*

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Corresponding Author: Rafaat M. Shaapan, Department of Zoonotic Diseases, Veterinary Research Division, National Research Center, P.O. Box 12622, El-Tahrir Street, Dokki, Giza, Egypt Tel: +00202-25272439 Fax: 00202-33371362

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cryptosporidiosis is a common gastrointestinal disorder in humans and animals caused by various *Cryptosporidium* species. The disease is self-limiting in the immune competent host but poses a significant threat in the immune deficient individuals[1,2]. *Cryptosporidium parvum* is the most common zoonotic species affecting a wide variety of mammals and this species accounts for the majority of water borne outbreaks[3,4]. The infectious stages of the organism (oocysts) are shed in the feces of affected individuals, survive in adverse environmental conditions and spread by direct contact or through contaminants (food, water). Due to the robustness of the oocysts, their tenacity, tiny size and resistance to common disinfectants, the parasite is difficult to eradicate from contaminated environments. To obtain sufficient control, both treatment of infected hosts and inactivation of oocysts are necessary[5]. Several drugs are commonly used to treat cryptosporidiosis in man and very few in animals but none of them are completely effective in terms of both clinical and parasitological response. Only a few chemical agents are able to inactivate oocysts in the environment including water treatment plants but their application has certain limitations. Therefore, control of cryptosporidiosis remains a global challenge in both veterinary and human medicine. Extensive research has been performed on suitable drugs and disinfectants. Thousands of agents have been tested both *in vivo* and *in vitro*. Some are excitingly active *in vitro* but exhibit poor or no response in clinical trials[5].

Many chemotherapeutic antimicrobial compounds have been tested, but most have only a partial effectiveness in the treatment and prophylaxis of cryptosporidiosis in animals and humans. There is no clearly recognized, widely accepted and immediately available compound that can be used as a prophylactic or therapeutic agent[6], despite some encouraging recent advances including the use of β-cyclodextrin[7], nitazoxanide[8], halofuginonelactate[9] and premomycin[10]. Because of the limited availability of effective drugs, hygienic measures and good management are currently the most valid weapons in controlling this disease. However, a number of studies have demonstrated the efficacy of oral administration of hyper immune, bovine colostrum in reducing the shedding of oocysts and the clinical signs of disease in lambs and calves[11].

Therefore, the objective of the current review article was to highlight and evaluate the new trends for control of cryptosporidiosis in animals and humans.

PREVENTION AND CONTROL

Treatment: Oral or intravenous fluid therapy remains the single most important treatment to diminish clinical signs of disease in humans and animals. Although more than 150 antimicrobial agents have been studied and a few have been demonstrated to reduce the magnitude of the symptoms, none eliminates the disease completely and none has received regulatory approval for the treatment of animal cryptosporidiosis. Trials evaluating different drugs, novel classes of compounds and immune therapy are currently in progress. Recently, the US FDA[12] approved the drug nitazoxanide (Alinia™) for the treatment of pediatric diarrhea caused by *C. parvum* in children 1-11 years of age. The efficacy of nitazoxanide (a synthetic antiprotozoal agent) in children was studied in double-blind placebo-controlled trials which showed reduced duration of diarrhea and oocyst excretion[13,14]. Nitazoxanide (NTZ) has been tested in animal models but showed only partial efficacy in the gnotobiotic pig model (with high doses, 150-250 mg kg^{-1} day^{-1}, reducing oocyst shedding) and no efficacy in the mouse model, bringing into question the true efficacy of the drug[15,16]. Aminoglycoside antibiotics, such as paromomycin have proven to decrease the patent period, oocyst shedding and clinical symptoms in mice and calves infected with *C. parvum* (Table 1)[15,17]. A recent report determined that dinitroanilineoryzalin reduced oocyst numbers and pathologic manifestations in the gut of mice infected with *C. parvum*[18]. The short term feeding (2-3 days) of relatively high levels (6-15 mg kg^{-1} day^{-1}) of the ionophore polyether antibiotic, lasalocid was reported to be an effective treatment for acute cryptosporidiosis in young calves[19] but was highly toxic when fed (at 8 mg kg^{-1} day^{-1}) for 2 weeks as a prophylaxis. Lower doses of Lasalocid have not demonstrated any efficacy against *C. parvum* infections. Awadalla[20] used sulphadimidine powder at a dose rate of 0.4 g Kg^{-1} b.wt., for treatment of cryptosporidiosis in diarrheic buffalo-calves and reported stoppage of diarrhea and improvement in the general health condition.

Alternative therapies such as passive immunotherapy using hyper immune, serum and hyper immune, bovine colostrum containing antibodies against *C. parvum* surface proteins as well as anti sporozoites monoclonal antibodies have also been tested with promising but inconclusive results[21,22]. Several colostrum preparations have been used to treat infections, the best results being obtained with hyper immune, bovine colostrum harvested from dairy

Table 1: Efficacy of different drugs against cryptosporidiosis in ruminants[29]

			Best results		
Drugs	Animal species	Dose*	Administration period	Oocysts shedding	Episode of diarrhoea# or stool consistence##
Halofuginone lactate	Lamb	500 µg	1-5 days	Decrease	Prevented#
Halofuginone lactate	Calf	30-500 µg	3-14 days	Decrease	Prevented#
Paromomycin	Calf	25-100 mg	11 days	Decrease	Reduced# improved##
Paromomycin	Goat kid	100 mg	12 days	Decrease	Improved##
Decoquinate	Calf	2.5-10 mg	8 weeks	Decrease	Improved##
Decoquinate	Goat kid	2.5 mg	21 days	Decrease	Prevented#

*Per kilogram body weight

cows vaccinated during gestation with *C. parvum* oocyst/sporozoites antigens. Treatment with hyper immune colostrum containing high levels of antibodies against *Cryptosporidium* (titers ranging from 1:3200-1:200000) has been associated with the symptomatic improvement or resolution of infection[23]. Laboratory studies have shown that prophylactic treatment with hyper immune colostrum partially protected calves and mice against experimental infection and clinical disease and incubation in hyper immune, colostrum has been found to neutralize sporozoites and reduced their infectivity[24]. Colostrum harvested from cows vaccinated by a combination of intramuscular injection and intra-mammary infusion was found to be effective in human and animal trials[25], whereas colostrum harvested from cows vaccinated by intramuscular injection alone did not protect calves against experimental infection despite the presence of specific antibodies at titer up to 1:10240. Another study, however, has found that colostrum harvested from cows following repeated parenteral immunization was effective in protecting calves against infection[24].

The administration of probiotics (live bacterial cell supplements) has been shown to reduce the duration and number of oocysts shed by experimentally infected mice[26,27]. In addition, *in vitro* studies have demonstrated that lactic acid bacteria supernatants significantly reduced oocyst viability[28]. Although bacterial mechanisms involved in protection against cryptosporidiosis are not identified, the studies suggest that probiotic bacteria may have potential for therapeutic use against *Cryptosporidium*.

Many other chemotherapeutic agents have been tested *in vitro* and *in vivo* for the treatment of cryptosporidiosis, but few agents have shown promise. Drugs such as decoquinate decrease oocyst shedding and improve the frequency and severity of diarrhea in calves, lambs and goat kids[10,29]. Halofuginone lactate (Halocur, Intervet) has recently been registered in Europe as a chemotherapeutic agent for cryptosporidiosis in domestic cattle, which has been shown to reduce incidence and severity of diarrhea[30]. It has also been reported to markedly reduce oocyst output in experimentally infected lambs and naturally infected calves (Table 1). The drug prevents reinfection of the gut by sporozoites and recycling merozoites. The preliminary studies suggest that an oral dose of 60-125 µg kg^{-1} b.wt., daily for 7 days will protect against clinical disease and will markedly reduce oocyst excretion but will allow some intestinal infections and thus the development of immunity[31]. The effectiveness of β-cyclodextrin in treatment of natural infection by *C. parvum* in suckling calves was also evaluated[7]. Administration of the drug at a dose of 50 mg kg^{-1} b.wt. for 3 consecutive days from birth (prophylactically) or following confirmation of the infection (therapeutically) decreased the severity of diarrhoea and shortened the duration of oocyst shedding. The efficacy of β-cyclodextrin demonstrated in this study suggests that it could be suitable for the use in treatment of cryptosporidiosis in dairy calves.

Affected calves should be treated with fluids and electrolytes both orally and parenterally as necessary until spontaneous recovery occurs. Cow's whole milk should be given in small quantities several times daily to optimize digestion and to minimize loss of body weight. It is important to continue to feed milk to the full level of requirement despite the presence of diarrhea, as a reduction in intake may lead to death from inanition. Several days of intensive care and feeding may be required before recovery is apparent. Parenteral nutrition could be considered for valuable calves[31].

Advances and prospects for subunit vaccines against cryptosporidiosis: Cryptosporidiosis causes self-limiting diarrhoea in humans and animals that resolves within 1-2 weeks post infection[23,32]. The disease is prevalent in the young and in immunocompromised animals and persons due to immature or impaired T cell immunity[33]. Prior exposure to *C. parvum* leads to increased, but not complete resistance to subsequent infection[34]. Protective immunity appears, based

on studies in humans and mice to involve CD4+T lymphocytes and associated the cytokines, IFN-γ and IL-12[35]. Although *C. parvum* specific IgG, IgA and IgM responses have been demonstrated, the role of these antibodies in resolution, parasite clearance and prevention of clinical signs remains unclear[36,37]. Cellular immunity could be more important for prevention of clinical signs and elimination of the parasite[30]. Vaccination has been proposed as a method to control cryptosporidiosis in animal populations[29,38]. The importance of colostrum in protecting neonatal ruminants against infection by *C. parvum* is a very valuable point to address. In field conditions, passively acquired antibodies did not protect calves[29,39] and lambs against naturally acquired infection[40]. However, both calves and lambs fed with colostrum from immunized mothers with high titres of specific antibodies were partially protected against infection[41]. Several studies have shown that passive immunotherapy with hyper immune, serum or colostrum against *C. parvum* antigens can ameliorate clinical signs of disease[38,42]. Vaccination against cryptosporidiosis has focused on antigens that may be used to generate colostrum for passive immunotherapy rather than stimulating protective immunity[29]. There are several reasons for this approach. First, vaccination of vulnerable individuals (the young) would probably not be effective during the period of peak susceptibility. Second, due to the sporadic occurrence of cryptosporidiosis, the age-related resistance to infection and since disease symptoms are rarely life-threatening, there is little perceived need for widespread vaccination. Vaccination of young animals with an immature immune system may not be effective and widespread vaccination may not be economical, as the disease is rarely lethal when good preventive measures are in place[19].

Immunodominant *Cryptosporidium* antigens have been identified from natural infections and subunit vaccines have been prepared and vaccination trials have been conducted in calves[30,38]. Using active and passive immunization approaches, vaccines have shown to reduce clinical signs but in most cases, have not eliminated or reduced oocyst shedding. Indeed, selection of immunodominant antigen-subunit vaccine candidates is based upon the premise that the T helper cell type 2 (Th 2) responses is responsible for eliminating the parasite and for protection from further infection[30].

Thus, cloning of *C. parvum* DNA has concentrated on expressing epitopes on the surface of invasive stages (sporozoites, merozoites) which may be involved in attachment to host cells of the intestinal epithelium. Although a number of recombinant *C. parvum* antigens have been demonstrated[43-47]. The greatest effort has been described at sequences coding for antigens related to sporozoites 15 or 23 kDa surface proteins[48-52]. Plasmid DNA expressing The CP 15/60 protein has been used to elicit serum and colostrum antibodies in sheep[42] and cows[53] against recombinant and native CP15 antigens. Treatment of immunosuppressed adult inbred mice with this anti-CP 15/60 hyper immune, bovine colostrum prior to and during experimental *C. parvum* oocyst infection hindered parasite development *in vivo*[53]. Although it is unknown whether direct plasmid DNA injection is required, a recent study showed that expression of CP15/60 in an eukaryotic vector (baculovirus) was superior to a prokaryotic system (*E. coli*) in terms of humoral and cellular responses elicited[54]. Nasal immunization of goats and mice with a second CP15 DNA sequence also led to anti-CP15 antibodies in serum that in the latter host species, remained high for at least 1 year post-immunization[55]. In mice, CP15-specific T cell proliferative responses were also demonstrated in both the spleen and the mesenteric lymph nodes[56]. Furthermore, kids challenged with a high number of *C. parvum* oocysts (10^6 oocysts per kid) and fed colostrum from CP15-immunized goats shed fewer oocysts and displayed less severe clinical effects compared to kids fed normal colostrum[55]. Another study showed that administration of monoclonal antibodies (mAbs) specific for a 23 kDa *C. parvum* sporozoites protein reduced the severity of *C. parvum* oocyst infection in mice[50]. Hyper immune, bovine colostrum prepared against recombinant CP23 antigen prevented diarrhea and reduced oocysts shedding in calves challenged with 10^6 *C. parvum* oocysts[36]. Thus, immunization of ruminants with either recombinant *C. parvum* sporozoites surface antigens or plasmid DNA encoding the CP15 or CP23 antigens appears to be a viable approach to produce colostrum for the passive immunotherapy of cryptosporidiosis.

Preventive hygienic measures: From a perspective of disease control, preventive hygienic measures are the most important tools in the struggle against cryptosporidiosis in farm animals, the objective being to destroy external forms of the parasite and to prevent their transmission among animals and from the environment to the host. Preventive measures are by far the most effective approach to control this parasite[19]. This was proven by the marked decline in human and animal cases of cryptosporidiosis during the 2001 epidemic of Foot and Mouth Disease (FMD) in UK. Limiting human access to the countryside, containment of animals and restriction of

livestock movement for trade or to pasture and extensive slaughtering of FMD-affected animals were the immediate actions taken that not only ended the FMD epidemic but also resulted in a significant reduction (81.8%) in reported cryptosporidiosis cases as compared to the previous year[57,58] 2000. These reports also provide clear evidence that zoonotic transmission is indeed a major route of human *Cryptosporidium* infection.

Because the major source of infection is contaminated water supplies, implementation of measures to decrease the spread of the parasitic oocysts in the environment is critical. Thus, identification of the risk factors for infection in livestock will allow for the logical development of oocyst shedding management strategies. Prevention of cryptosporidiosis transmissions is dependent on hygienic measures in any setting. The destruction of oocysts on surfaces of housing facilities is possible by cleaning with 5% ammonia solutions[30], especially if heat can also be applied. Isolation of ill animals and ensuring that newborns have received adequate colostrum are always good preventive actions to control infectious diseases. Although passively acquired antibodies have not been effective in protecting calves against *Cryptosporidium* infections, calves fed with hyper immune, , colostrum from immunized dams developed less severe diarrhea and shed fewer oocysts than calves fed "nonhyper immune, " colostrum[19].

Measures that reduce transmission between animals should be encouraged. Limiting the number of animals enclosed in the same facilities (i.e. reduced stocking density), keeping young animals separated from adults, minimizing contact between personnel and calves and maintaining a short calving period may assure reduced opportunities for the parasite to spread within a herd. Reproductive management such as increasing herd fertility and increasing the bull: cow ratio, limiting the time of cow and bull exposure may help reduce the calving period while maintaining calving rates[59]. In ruminant husbandry, the destruction of oocysts in the pens and building used for parturition by applying moist heat and/or chemical disinfection, the use of abundant clean straw beds, avoidance of high stocking rates in the parturition area and the separation of healthy and ill animals during outbreaks of diarrhoea, in addition to the administration of appropriated supplies of colostrum to neonates, all help to prevent outbreaks of cryptosporidiosis and to minimize mortality and morbidity in infected herds[29].

Many recommendations have been made for the prevention and control of infections in specific locations; such as hospitals, laboratories, households, zoos and farms. These recommendations have basically involved managerial practice designed to minimize further host contact with sources of infection and the use of different disinfection procedures to destroy infective oocysts. Infected individuals should be identified and isolated to confine infections to particular areas which can be regularly cleaned and disinfected. Susceptible individuals should avoid contact with contaminated areas or to be removed to safe locations. Care should be exercised in the handling and disposal of biohazardous waste and suspected contaminated water should be boiled prior to consumption or use. *Cryptosporidium* species can be removed from drinking water by either boiling for 1 min or filtering through a filter with a pore size of less than 1 μm[60]. Immunosuppressed persons should avoid contact with animals with diarrhea, dogs or cats younger than 6 months of age and stray animals[61]. The examination of an animal's stool by the veterinarian before the person has contact with the animal is critical for those at risk of infection.

Laboratory studies have shown that oocysts stored at aqueous solutions have remained viable for up to 3 months at ambient temperatures (15-20°C) and for up to one year when refrigerated (4-6°C). Infectivity was lost after oocysts had been heated to 65°C for at least 30 min or when desiccated for at least 4 h (Table 2). Snap-freezing has been shown to kill oocysts, whereas slow freezing was less effective and some oocycts have survived freezing at -22°C for up to 1 month[62].

Like many other coccidian oocysts, those of *Cryptosporidium* have proven remarkably resistant to chemical disinfection. Laboratory studies have shown that many commercial disinfectants (based on aldehyde, ammonia,

Table 2: Disinfection procedures reportedly effective against *Cryptosporidium* oocysts[32]

Agents	Applications
Ammonia	5% for 120 min
	50% for 30 min
Chlorine dioxide	0.4 ppm for 15 min
	1.3 ppm for 60 min
Dessication	Air-drying, 4 h
Exspor*	Working dilution 30 min
Formol saline	10% for 120 min
Freezing	-70°C, time unspecified
Heat	65°C for 30 min
Hydrogen peroxide	3% (10 vol) for 30 min
Moist heat	45-55°C for 15-20 min
Oo-cide**	5% for 5 min
Ozone	1.11 ppm for 6 min
	1 ppm for 5 min

*Chlorine dioxide-based cold sterilant (Alcide Co.), **Two-phase product producing ammonia (Antec International Ltd)

alcohol, chlorine or alkaline compounds) are ineffective when used according to the manufacturer's instructions. In some cases, higher concentrations and longer exposure periods have been found to kill oocysts but the prolonged use of concentrated disinfectants in confined areas is inadvisable or impractical in many situations. To date, over 35 disinfectants have been tested but only 5 have been found to be effective following relatively short exposure periods; 50% ammonia, 3% hydrogen peroxide, 10% formalin, Exspor and Oo-cide (Table 2). Steam heat sterilization and fumiogation with formaldehyde or ammonia gas have also been recommended as appropriate forms of decontamination[23,63].

Recent studies have indicated that most conventional methods of water treatment do not effectively remove or kill all *Cryptosporidium* oocysts from contaminated water. Laboratory simulation of different water treatment processes has shown that some oocysts can survive treatment with chlorine, monochloramine, lime, polyelectrolytes, alum and ferric sulphate[64]. Because chlorination does not inactivate *Cryptosporidium*, alternative disinfectants such as ozone and UV radiation have being evaluated. Low doses of UV (1-9 mJ cm^{-2}) can inactivate 2-4 log_{10} (99-99.9%) of *C. parvum* oocysts[65] and low doses (2 mg L^{-1}) of ozone for short times (1 min) are able to inactive up to 99% of oocysts[66]. The percent inactivation rate of free chlorine dioxide disinfection for *Cryptosporidium* is 90-97% using higher level (60-80 mg for 1 min L^{-1})[64]. Quantitative studies on the treatment of wastewater and sewage have shown that 74-84% of oocysts are removed by activated-sludge systems and 87-99% by subsequent sand filtration[67]. Sand filters used to treat drinking water and swimming pools have been found to remove 91% of oocysts[68]. Alternative to conventional water treatment, pressure-driven membrane processes, microfiltration (0.1-10 μM pore membrane), ultrafiltration (0.002-0.1 μM pore membrane), nano filtration and Reverse Osmosis (RO) have proven complete removal of all protozoan cysts[69,70]. As a result, these membrane technologies are playing a major role in drinking water production in the US and Europe[71]. In the US, the Centers for Disease Control and prevention (CDC) reported that only RO or less than 1 micron filters are effective for the removal of *Cryptosporidium* oocysts and advise the exclusive use of bottled water processed under those treatments[72].

CONCLUSION

Preventive hygienic measures are by far the most effective approach to control this parasite in farm animals, the objective being to destroy the external forms of the parasite (infective mature oocysts) and to prevent their transmission among animals. The destruction of oocysts on surfaces of housing facilities, pens and parturition buildings is possible using effective disinfectants such as 50% ammonia, 3% hydrogen peroxide or 10% formalin. Measures to reduce transmission between animals should be encouraged. Limiting the number of animals enclosed in the same facilities and avoidance of high stocking rates in the parturition area, maintaining a short calving period, administration of appropriated supplies of colostrum especially hyper immune, colostrum from immunized dams, isolation and treatment of diarrheic infected animals; all help to prevent outbreaks of cryptosporidiosis, reduce the spread of infection within a herd and minimize mortality and morbidity in infected herds. Chemotherapeutic agents such as paromomycin, decoquinate, lasalocid, halofuginone lactate, nitazoxanide, dinitrooryzalin, β-cyclodextrin and probioties have proved a potential therapeutic effect against cryptosporidiosis in the form of reduction the duration and numbers of oocysts shedding and the incidence and severity of the diarrhea. Immunization of ruminants during pregnancy with either recombinant *C. parvum* sporozoites surface antigens or plasmid DNA encoding the CP15 or CP23 antigens appear to be a valuable approach for producing colostrum for the passive immunotherapy of cryptosporidiosis. Hyper immune, colostrum prevented diarrhea and reduced oocysts shedding in newly born calves, lambs and goat kids exposed to *Cryptosporidium* infections.

REFERENCES

1. Alves, M., O. Matos, I.P. da Fonseca, E. Delgado, A.M. Lourenco and F. Antunes, 2001. Multilocus genotyping of *Cryptosporidium* isolates from human HIV-infected and animal hosts. J. Eukaryotic Microbiol., 48: 17s-18s.
2. Mohandas, K., R. Sehgal, A. Sud and N. Malla, 2002. Prevalence of intestinal parasitic pathogens in HIV-Seropositive individuals in Northern India. Jap. J. Infect. Dis., 55: 83-84.
3. Karanis, P., C. Kourenti and H. Smith, 2007. Waterborne transmission of protozoan parasites: A worldwide review of outbreaks and lessons learnt. J. Water Health, 5: 1-38.
4. Baldursson, S. and P. Karanis, 2011. Waterborne transmission of protozoan parasites: Review of worldwide outbreaks: An update 2004-2010. Water Res., 45: 6603-6614.
5. Shahiduzzaman, M. and A. Daugschies, 2012. Therapy and prevention of cryptosporidiosis in animals. Vet. Parasitol., 188: 203-214.
6. Coombs, G.H. and S. Muller, 2002. Recent advances in the search for new anti-coccidial drugs. Int. J. Parasitol., 32: 497-508.

7. Castro-Hermida, J.A., Y. Gonzalez-Losada, F. Freire-Santos, M. Mezo-Menendez and E. Ares-Mazas, 2001. Evaluation of β-cyclodextrin against natural infections of cryptosporidiosis in calves. Vet. Parasitol., 101: 85-89.
8. Gilles, H.M. and P.S. Hoffman, 2002. Treatment of intestinal parasitic infections: A review of nitazoxanide. Trends Parasitol., 18: 95-97.
9. Villacorta, I., J.E. Peeters, E. Vanopdenbosch, E. Ares-Mazas and H. Theys, 1991. Efficacy of halofuginone lactate against *Cryptosporidium parvum* in calves. Antimicrob. Agents Chemother., 35: 283-287.
10. Grinberg, A., A. Markovics, J. Galindez, N. Lopez-Villalobos, A. Kosak and V.M. Tranquillo, 2002. Controlling the onset of natural cryptosporidiosis in calves with paromomycin sulphate. Vet. Rec., 151: 606-608.
11. Martin-Gomez, S., M.A. Alvarez-Sanchez and F.A. Rojo-Vazquez, 2005. Immunization protocols against *Cryptosporidium parvum* in ovines: Protection in suckling lambs. Vet. Parasitol., 129: 11-20.
12. FDA., 2002. FDA approves new treatment for parasitic infections in pediatric patients. FDA Talk Paper, December 2, 2002, U.S. Food and Drug Administration (FDA), Silver Spring, MD., USA.
13. Rossignol, J.F.A., A. Ayoub and M.S. Ayers, 2001. Treatment of diarrhea caused by *Cryptosporidium parvum*: A prospective randomized, double-blind, placebo-controlled study of nitazoxanide. J. Infectious Dis., 184: 103-106.
14. Amadi, B., M. Mwiya, J. Musuku, A. Watuka, S. Sianongo, A. Ayoub and P. Kelly, 2002. Effect of nitazoxanide on morbidity and mortality in Zambian children with cryptosporidiosis: A randomised controlled trial. Lancet, 360: 1375-1380.
15. Blagburn, B.L., K.L. Drain, T.M. Land, R.G. Kinard and P.H. Moore *et al*., 1998. Comparative efficacy evaluation of dicationic carbazole compounds, nitazoxanide and paromomycin against *Cryptosporidium parvum* infections in a neonatal mouse model. Antimicrob. Agents Chemother., 42: 2877-2882.
16. Theodos, C.M., J.K. Griffiths, J. D'Onfro, A. Fairfield and S. Tzipori, 1998. Efficacy of nitazoxanide against *Cryptosporidium parvum* in cell culture and in animal models. Antimicrob. Agents Chemother., 42: 1959-1965.
17. Fayer, R. and W. Ellis, 1993. Paromomycin is effective as prophylaxis for cryptosporidiosis in dairy calves. J. Parasitol., 79: 771-774.
18. Armson, A., K. Menon, A. O'Hara, L.M. MacDonald and C.M. Read *et al*., 2002. Efficacy of oryzalin and associated histological changes in *Cryptosporidium*-infected neonatal rats. Parasitology, 125: 113-117.
19. Ramirez, N.E., L.A. Ward and S.A. Sreevatsan, 2004. A review of the biology and epidemiology of cryptosporidiosis in humans and animals. Microb. Infect., 6: 773-785.
20. Awadalla, S.A.M., 1996. Studies on enteritis in newly born buffalo-calves. Ph.D. Thesis, Faculty of Veterinary Medicine, Zagazig University, El Sharkia, Egypt.
21. Hunt, E., Q. Fu, M.U. Armstrong, D.K. Rennix and D.W. Webster *et al*., 2002. Oral bovine serum concentrate improves cryptosporidial enteritis in calves. Pediatric Res., 51: 370-376.
22. Riggs, M.W., D.A. Schaefer, S.J. Kapil, L. Barley-Maloney and L.E. Perryman, 2002. Efficacy of monoclonal antibodies against defined antigens for passive immunotherapy of chronic gastrointestinal cryptosporidiosis. Antimicrob. Agents Chemother., 46: 275-282.
23. O'Donoghue, P.J., 1995. *Cryptosporidium* and cryptosporidiosis in man and animals. Int. J. Parasitol., 25: 139-195.
24. Doyle, P.S., J. Crabb and C. Petersen, 1993. Anti-*Cryptosporidium parvum* antibodies inhibit infectivity *in vitro* and *in vivo*. Infect. Immun., 61: 4079-4084.
25. Fayer, R., J.R. Barta, A.J. Guidry and B.L. Blagburn, 1991. Immunogold labeling of stages of *Cryptosporidium parvum* recognized by immunoglobulins in hyperimmune bovine colostrum. J. Parasitol., 77: 487-490.
26. Alak, J.I.B., B.W. Wolf, E.G. Mdurvwa, G.E. Pimentel-Smith and O. Adeyemo, 1997. Effect of *Lactobacillus reuteri* on intestinal resistance to *Cryptosporidium parvum* infection in a murine model of acquired immunodeficiency syndrome. J. Infect. Dis., 175: 218-221.
27. Alak, J.I., B.W. Wolf, E.G. Mdurvwa, G.E. Pimentel-Smith, S. Kolavala, H. Abdelrahman and V. Suppiramaniam, 1999. Supplementation with *Lactobacillus reuteri* or *L. acidophilus* reduced intestinal shedding of *Cryptosporidium parvum* oocysts in immunodeficient C57BL/6 mice. Cell. Mol. Biol., 45: 855-863.
28. Foster, J.C., M.D. Glass, P.D. Courtney and L.A. Ward, 2003. Effect of Lactobacillus and Bifidobacterium on *Cryptosporidium parvum* oocyst viability. Food Microbiol., 20: 351-357.
29. De Graaf, D.C., E. V anopdenbosch, L.M. Ortega-Mora, H. Abbassi and J.E. Peeters, 1999. A review of the importance of cryptosporidiosis in farm animals. Int. J. Parasitol., 29: 1269-1287.
30. Olson, M.E., R.M. O'Handley, B.J. Ralston, T.A. McAllister and R.C.A. Thompson, 2004. Update on *Cryptosporidium* and *Giardia* infections in cattle. Trends Parasitol., 20: 185-191.
31. Radostits, O.M., D.C. Blood and C.C. Gay, 1994. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 8th Edn., B. Saunders Co. Ltd., London pp: 1195-1199.
32. Fayer, R., C.A. Speer and J.P. Dubey, 1997. The General Biology of *Cryptosporidium*. In: *Cryptosporidium* and Cryptosporidiosis, Fayer, R. (Ed.). CRC Press, Boca Raton, USA., pp: 1-42.

33. Casemore, D.P., S.E. Wright and R.L. Coop, 1997. Cryptosporidiosis: Human and Animal Epidemiology. In: *Cryptosporidium* and Cryptosporidiosis, Fayer, R. (Ed.). CRC Press, Boca Raton, FL., pp: 65-92.
34. Chappell, C.L., P.C. Okhuysen, C.R. Sterling, C. Wang, W. Jakubowski and H.L. Dupont, 1999. Infectivity of *Cryptosporidium parvum* in healthy adults with pre-existing anti-*C. parvum* serum immunoglobulin G. Am. J. Trop. Med. Hyg., 60: 157-164.
35. Riggs, M.W., 1998. Immunology: Host Response and Development of Passive Immunotherapy and Vaccines. In: *Cryptosporidium* and cryptosporidiosis, Fayer, R. (Ed.). CRC Press, Boca Raton, Florida, USA, pp: 129-162.
36. Perryman, L.E., S.J. Kapil, M.L. Jones and E.L. Hunt, 1999. Protection of calves against cryptosporidiosis with immune bovine colostrum induced by a *Cryptosporidium parvum* recombinant protein. Vaccine, 17: 2142-2149.
37. Riggs, M.W., 2002. Recent advances in cryptosporidiosis: The immune response. Microbes Infect., 4: 1067-1080.
38. Jenkins, M.C., 2001. Advances and prospects for subunit vaccines against protozoa of veterinary importance. Vet. Parasitol., 101: 291-310.
39. Peeters, J.E., I. Villacorta, E. Vanopdenbosch, D. Vandergheynst, M. Naciri, E. Ares-Mazas and P. Yvore, 1992. *Cryptosporidium parvum* in calves: Kinetics and immunoblot analysis of specific serum and local antibody responses (immunoglobulin A [IgA], IgG and IgM) after natural and experimental infections. Infect. Immun., 60: 2309-2316.
40. Ortega-Mora, L.M., J.M. Troncoso, F.A. Rojo-Vazquez and M. Gomez-Bautista, 1993. Serum antibody response in lambs naturally and experimentally infected with *Cryptosporidium parvum*. Vet. Parasitology, 50: 45-54.
41. Naciri, M., R. Mancassola, J.M. Reperant, O. Canivez, B. Quinque and P. Yvore, 1994. Treatment of experimental ovine cryptosporidiosis with ovine or bovine hyperimmune colostrum. Vet. Parasitol., 53: 173-190.
42. Jenkins, M., D. Kerr, R. Fayer and R. Wall, 1995. Serum and colostrum antibody responses induced by jet-injection of sheep with DNA encoding a *Cryptosporidium parvum* antigen. Vaccine, 13: 1658-1664.
43. Lally, N.C., G.D. Baird, S.J. McQuay, F. Wright and J.J. Oliver, 1992. A 2359-base pair DNA fragment from *Cryptosporidium parvum* encoding a repetitive oocyst protein. Mol. Biochem. Parasitol., 56: 69-78.
44. Spano, F., C. Puri, L. Ranucci, L. Putignani and A. Crisanti, 1997. Cloning of the entire COWP gene of *Cryptosporidium parvum* and ultrastructural localization of the protein during sexual parasite development. Parasitology, 114: 427-437.
45. Barnes, D.A., A. Bonnin, J.X. Huang, L. Gousset and J. Wu *et al*., 1998. A novel multi-domain mucin-like glycoprotein of *Cryptosporidium parvum* mediates invasion. Mol. Biochem. Parasitol., 96: 93-110.
46. Tosini, F., S. Caccio, A. Tamburrini, G. La Rosa and E. Pozio, 1999. Identification and characterisation of three antigenic proteins from *Cryptosporidium parvum* sporozoites using a DNA library expressing poly-histidine tagged peptides. Int. J. Parasitol., 29: 1925-1933.
47. Priest, J.W., J.P. Kwon, M.J. Arrowood and P.J. Lammie, 2000. Cloning of the immunodominant 17-kDa antigen from *Cryptosporidium parvum*. Mol. Biochem. Parasitol., 106: 261-271.
48. Jenkins, M.C., P.G. Seferian, P.C. Augustine and H.D. Danforth, 1993. Protective immunity against coccidiosis elicited by radiation-attenuated *Eimeria maxima* sporozoites that are incapable of asexual development. Avian Dis., 37: 74-82.
49. Jenkins, M.C., R. Fayer, M. Tilley and S.J. Upton, 1993. Cloning and expression of a cDNA encoding epitopes shared by 15-and 60-kilodalton proteins of *Cryptosporidium parvum* sporozoites. Infect. Immunity, 61: 2377-2382.
50. Perryman, L.E., D.P. Jasmer, M.W. Riggs, S.G. Bohnet, T.C. McGuire and M.J. Arrowood, 1996. A cloned gene of *Cryptosporidium parvum* encodes neutralization-sensitive epitopes. Mol. Biochem. Parasitol., 80: 137-147.
51. Khramtsov, N.V., B. Oppert, B.A. Montelone and S.J. Upton, 1997. Sequencing, analysis and expression in *Escherichia coli* of a gene encoding a 15 kDa *Cryptosporidium parvum* protein. Biochem. Biophys. Res. Commun., 230: 164-166.
52. Cevallos, A.M., X. Zhang, M.K. Waldor, S. Jaison and X. Zhou *et al*., 2000. Molecular cloning and expression of a gene encoding *Cryptosporidium parvum* glycoproteins gp40 and gp15. Infect. Immun., 68: 4108-4116.
53. Jenkins, M.C., C. O'Brien, J. Trout, A. Guidry and R. Fayer, 1999. Hyperimmune bovine colostrum specific for recombinant *Cryptosporidium parvum* antigen confers partial protection against cryptosporidiosis in immunosuppressed adult mice. Vaccine, 17: 2453-2460.
54. Iochmann, S., S. Sagodira, M .N. Mevelec, J.M. Reperant, M. Naciri, P. Coursaget and D. Bout, 1999. Comparison of the humoral and cellular immune responses to two preparations of *Cryptosporidium parvum* CP15/60 recombinant protein. Microb. Pathogen., 26: 307-315.
55. Sagodira, A., D. Buzoni-Gatel, S. Iochmann, M. Naciri and D. Bout, 1999. Protection of kids against *Cryptosporidium parvum* infection after immunization of dams with CP15-DNA. Vaccine, 17: 2346-2355.
56. Sagodira, S., S. Iochmann, M.N. Mevelec, I. Dimier-Poisson and D. Bout, 1999. Nasal immunization of mice with *Cryptosporidium parvum* DNA induces systemic and intestinal immune responses. Parasite Immunol., 21: 507-516.
57. Smerdon, W.J., T. Nichols, R.M. Chalmers, H. Heine and M. Reacher, 2003. Foot and mouth disease in livestock and reduced cryptosporidiosis in humans, England and Wales. Emerg. Infect. Dis., 9: 22-28.

58. Hunter, P.R., R.M. Chalmers, Q. Syed, L.S. Hughes, S. Woodehouse and L. Swift, 2003. Foot and mouth disease and cryptosporidiosis: Possible interaction between two emerging infectious diseases. Emerg. Infect. Dis., 9: 109-112.
59. Hoar, B.R., E.R. Atwill, C. Elmi and T.B. Farver, 2001. An examination of risk factors associated with beef cattle shedding pathogens of potential zoonotic concern. Epidemiol. Infect., 127: 147-155.
60. Leav, B.A., M. Mackay and H.D. Ward, 2003. *Cryptosporidium* species: New insights and old challenges. Clin. Infect. Dis., 36: 903-908.
61. Juranek, D.D., 1995. Cryptosporidiosis: Sources of infection and guidelines for prevention. Clin. Infect. Dis., 21: S57-S61.
62. Robertson, L.J., A.T. Campbell and H.V. Smith, 1992. Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. Applied Environ. Microbiol., 58: 3494-3500.
63. Fayer, R., C.A. Speer and J.P. Dubey, 1990. General Biology of *Cryptosporidium*. In: Cryptosporidiosis of Man and Animals, Dubey, J.P., C.A. Speer and R. Fayer (Eds.). CRC Press, Boston, pp: 1-29.
64. Rose, J.B., J.T. Lisle and M. LeChevallier, 1997. Waterborne Cryptosporidiosis: Incidence, Outbreaks and Treatment Strategies. In: *Cryptosporidium* and cryptosporidiosis, Fayer, R. (Ed.). CRC Press, Boca Raton, New York, pp: 93-109.
65. Craik, S.A., D. Weldon, G.R. Finch, J.R. Bolton and M. Belosevic, 2001. Inactivation of *Cryptosporidium parvum* oocysts using medium-and low-pressure ultraviolet radiation. Water Res., 35: 1387-1398.
66. Corona-Vaszuez, B., A. Samuelson, J.L. Rennecker and B.J. Marinas, 2002. Inactivation of *Cryptosporidium parvum* oocysts with ozone and free chlorine. Water Res., 36: 4053-4063.
67. De Maturana, I.V.M., M.E. Ares-Mazas, D. Duran-Oreiro and M.J. Lorenzo-Lorenzo, 1992. Efficacy of activated sludge in removing *Cryptosporidium parvum* oocysts from sewage. Applied Environ. Microbiol., 58: 3514-3516.
68. Chapman, R.A. and B.A. Rush, 1990. Efficiency of sand filtration for removing *Cryptosporidium* oocysts from water. J. Med. Microbiol., 32: 243-245.
69. Jacangelo, J.G., S.S. Adham and J.M. Laine, 1995. Mechanism of *Cryptosporidium*, *Giardia* and MS2 virus removal by MF and UF. J. Am. Water Works Assoc., 87: 107-121.
70. Horman, A., R. Rimhanen-Finne, L. Maunula, C. von Bonsdorff, J. Rapala, K. Lahti and M. Hnninen, 2004. Evaluation of the purification capacity of nine portable, small-scale water purification devices. Water Sci. Technol., 50: 179-183.
71. Betancourt, W.Q. and J.B. Rose, 2004. Drinking water treatment processes for removal of *Cryptosporidium* and *Giardia*. Vet. Parasitol., 126: 219-234.
72. CDC., 1997. *Cryptosporidium* and Water: A Public Health Handbook. Center for Disease Control and Prevention, Atlanta, GA., USA.