A Review On Cryptosporidiosis In Calves

Yilma Mulushet, Askale Abrhaley and Basaznew Bogale

College Of Veterinary Medicine And Animla Science, Faculty Of Veterinary Medicine, University Of Gondar, P.O. Box 196, Gondar, Ethiopia
Corresponding authors: Yilma Mulushet, e-mail: ymulushet@gmail.com

Summary: The genus *Cryptosporidium* is the most common cause of morbidity and mortality in preweaned dairy calves worldwide. It is a very important genus that causes watery diarrhea in young, unweaned mammalian livestock. *Cryptosporidium* is now regarded as an economically important cause of neonatal diarrhea in calves and lambs. Due to the presence of multiple transmission routes and host range, the epidemiology of cryptosporidiosis is complex. One major problem in understanding the transmission of *Cryptosporidium* infection is the lack of morphologic features that clearly differentiate one *Cryptosporidium* spp. from many others. This features also results in difficulty of diagnostic identification of the parasite in fresh sample, unless concentration and staining methods are hold on. Prevention of calf diarrhea is difficult because of the large number of pathogens that may be involved. Different management and environmental factors have also been associated with the disease. When treatment is indicated, no safe and effective therapy for cryptosporidial enteritis has been successfully developed, but supportive care is the only treatment for the illness. The objective of the review is focused on the control of *Cryptosporidium* by combining a good hygiene management and effective preventive drugs and its potential risk factor due to the presence of a large range and abundance of animal reservoirs, mainly in young farmed animals.
[Yilma Mulushet, Askale Abrhaley and Basaznew Bogale. **A Review On Cryptosporidiosis In Calves.** *Nat Sci* 2017;15(2):68-75]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 11. doi:10.7537/marsnsj150217.11.

Key words: *Cryptosporidium*, *Oocyst*

1. Introduction

Cryptosporidium is a coccidian protozoan parasite that infects the microvillus border of the gastrointestinal epithelium of a wide range of vertebrate hosts, including humans. It is an obligate intracellular parasite of man and other mammals, birds, reptiles and fish. For several decades, *Cryptosporidium* was thought to be a rare, opportunistic animal pathogen, but after a time, *Cryptosporidium* spp. was reported by Tyzzer (1907) as infective in mice, and then it was identified as first human case noted in 1976 (Flanigan, 1993).

The cause of the cryptosporidiosis is highly dependent on the immune status of the host. While in immunocompetent individuals *Cryptosporidium* infections most commonly result in acute self-limiting gastroenteritis, in immunocompromised individuals it develops a chronic and life-threatening diarrheal disease (Chen, 2002). Due to their immature immune status, neonates are highly susceptible for infections with *Cryptosporidium* and routinely get infected by oral uptake of even low infective doses of the parasite's oocysts. Cryptosporidiosis has a higher incidence in developing countries, especially in malnourished and patient children. *Cryptosporidium* mostly infects children less than five years old and peaks for children less than two years old. In industrialized countries, cryptosporidiosis also occurs in adults due to food borne or waterborne outbreaks which results in diarrhea (Desai *et al*., 2012).

Control of cryptosporidiosis has to rely on reducing the prevalence of the parasite and on breaking the transmission pathways of *Cryptosporidium* species. The epidemiological information such as, the magnitude of infections, the spatial distribution of species and in risk groups of animals are important for planning of control measures. Swimming pools and recreational water use as well as tap water is associated with sporadic outbreaks of cryptosporidiosis. Filtering will prevent ingestion of *Cryptosporidia* from tap water (Addiss, 1993).

The *Cryptosporidium* is now increasingly considered an important foodborne and waterborne pathogen causing a disease of socioeconomic and public health significance worldwide. In humans the disease results in sickness and severe diarrhea and can be life threatening in the very young, elderly and in immunosuppressed individuals. Various community outbreaks due to contamination of water have a great public health importance (Karanis *et al*., 2007). Therefore, the objectives of this seminar are:

- To review on *Cryptosporidium* infection in calves.
- To identify the potential risk factors of *Cryptosporidium* infection related to its prevalence rate in calves.

- To recommend possible prevention and control means.

2. Literature Review

3. Cryptosporidiosis

Cryptosporidiosis is a diarrheal disease caused by an obligate intracellular protozoan parasite. *Cryptosporidium* can live in the intestine of humans and animals and is passed in the stool of an infected person or animal. Both the disease and the parasite are also known as "Crypto." The parasite has an outer shell that helps to stay in external environment for long period of time and makes it very resistant to chlorine-based disinfectants. The parasite is found in every region of the world (Scallan, 2011).

3.1. Etiology

Nowadays, more than 20 species of *Cryptosporidium* have been described, out of these only 6 are accepted as valid. *Cryptosporidium parvum* have the potential to cause diarrhea in both humans and other mammals (Lendner *et al*., 2011). Additional species names have been given when isolated from different hosts, namely: *Cryptosporidium hominis* found primarily in humans, *C. parvum*, found in humans and other mammals, *C. andersoni* and *C. bovis* in cattle, *C. canis* in dogs, *C. muris* in mice, *C. felis* in cats, *C. wrairi* in guinea-pigs, and *C. suis* in pigs. In livestock, *C. parvum*, *C. andersoni*, *C. bovis and C. ryanae* affects mammals and species like *C. galli, C. baileyi* and *C. meleagridis* have been reported to cause morbidity and outbreaks of disease in poultry, whereas *C. serpentis* and *C. varanii* are the cause in reptiles (Barriga, 1997).

3.2. Epidemiology

The occurrence of *Cryptosporidium* is worldwide. In developing countries the prevalence ranges from 3% to 20%. Many reports associate infection in calves with diarrhea occurring 5-15 days of age. *Cryptosporidium parvum* is often the only pathogen found in diarrhoeic calves (Singh *et al*., 2005). The most well-known epidemic occurred in 1993 in Milwaukee, Wisconsin, US. *Cryptosporidium parvum* infects numerous mammals in addition to humans. The occurrence is very high in unweaned animals. Subsequently, the infection has been found in up to 80% of calves less than 1 month of age and up to 62% of apparently healthy adult cattle (Tzipori *et al*., 1983).

The source of infection is animal or human fecal matter that contains oocysts. Fertilizing salad vegetables with manure is known to be a source of human infection. Young animals are the principal source of oocysts for the environment. Adult ruminants also excrete *Cryptosporidium* oocysts. The rates of prevalence in those adult animals excrete reach approximately 100%. Thus, the role of adult ruminants as a parasite reservoir gives the high prevalence of infection of herds and individual animals. For ewes and does, oocyst excretion increase during parturition. The high density of sheep and goats in watersheds and the excretion of high numbers of oocysts make these animal important sources of *Cryptosporidium* (Anderson, 1998).

Cryptosporidium can be transmitted through direct or indirect contact with faeces of the shedders. Indirect transmission happens when the feces containing *Cryptosporidium* oocyst contaminates materials, including water, food, and fomites such as clothes and footwear. It also occurs through environmental contamination, usually involving the release of feces, sewage, wastewater, often as overflow following heavy rain events, while direct transmission occurs by the fecal-oral route from infected hosts, including animal to animal, animal-to-human (zoonotic), human-to-animal, and human-to-human (anthroponotic) transmissions (Xiao, 2000).

Human-to-human transmission: *Cryptosporidium* is easily transmitted among children and staff members in day-care centers, and the spread of these outbreaks in the households of the attending children; share toilets and common play areas, or necessitate frequent diaper-changing, but the major risk factors are household contacts with people especially, children with diarrhea (Keusch *et al*., 1995). There have been also several reports of both transmission from patients to health care staff and patient-to-patient transmission. Patient-to-patient or patient-to- health care staff transmission may occur in hospitals due to poor diaper-changing and hand-washing practices by caregivers (Pandak, 2006).

Animal-to-human transmission: Although the transmission of *C. parvum* from household pets is extremely rare, it is the most important zoonotic agent of cryptosporidiosis, with a large range and abundance of animal reservoirs, mainly in young farmed animals. Approximately 50% of calves shed oocysts and the pathogen is present on upwards of 90% of all dairy farms. The high prevalence of the *C. parvum* in cattle and sheep and the high numbers of oocysts shed by infected animals especially, newborns make them important sources of environmental contamination with *Cryptosporidium* oocysts that are able to infect humans. Humans having direct interaction with infected animals especially, calves during management have a wide probability of getting infection. Zoonotic transmission may also occur through food and drinks (raw meat and milk, farm-made apple cider) (Current, 1994; Casemore *et al*., 1997).

Cryptosporidium was isolated from 152 species of mammals. This indicates that, the host-range of *C.*

parvum is very broad and expanded through a wide variety of domestic and wild animals. Among domestic animals, the frequency of *Cryptosporidium parvum* is high in cattle, mainly in calves. Concerning wildlife, the prevalence of the parasite is high in deer and some rodents (Casemore *et al.*, 1997).

Table 1. Valid *Cryptosporidium* species

Species	Major host	Minor host
C. muris	Rodents, bactrian camels	Humans, rock hyrax, mountain goat
C. andersoni	Cattle, Bactrian Camels	Sheep
C. parvum	Cattle, sheep, goats, Humans	Deer, mice, pigs
C. hominis	Humans, monkeys	Dugongs, sheep
C. wrairi	Guinea, pigs	
C. felis	Cats	Humans, cattle
C. canis	Dogs	Humans
C. meleagridis	Turkeys, humans	Parrots
C. baileyi	Chicken, turkeys	Cockatiels, quails, ostriches, ducks
C. scophithalmi	Fish	
C. suis	Pigs	Humans

Source: Xiao *et al.*, 2004

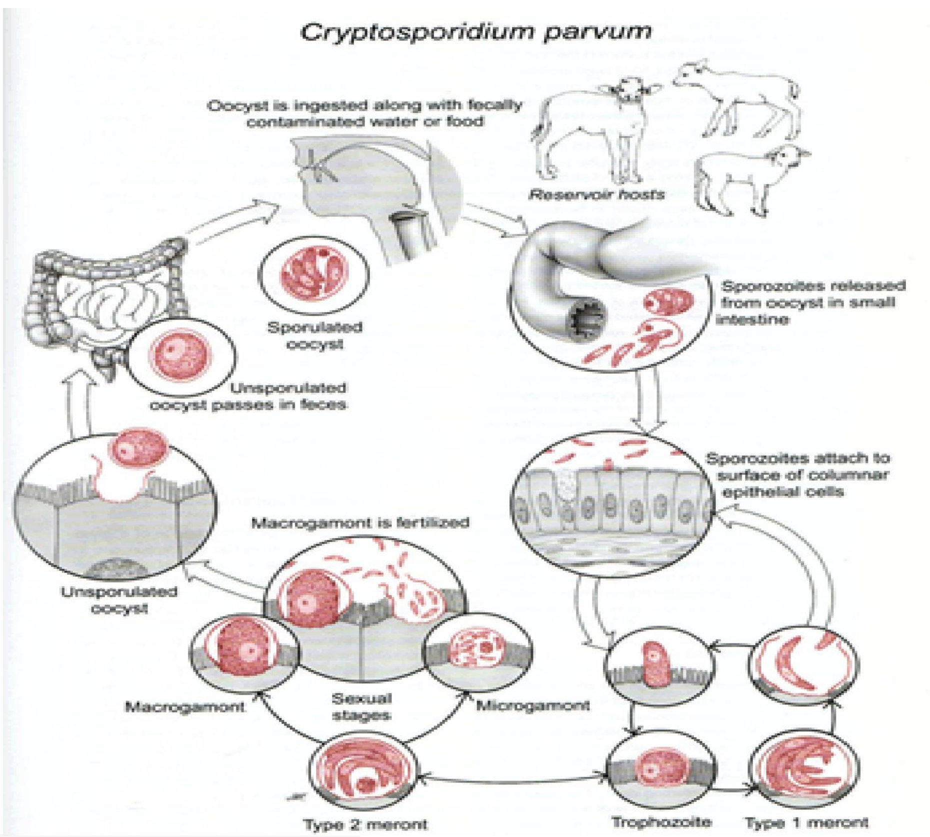

Figure 1: life cycle of *Cryptosporidium parvum* (http://www.hoards.com/IB_MGB-*C-parvum*) [accessed on 10 April 2016]

3.3. Life cycle

The life cycle of *Cryptosporidium* is direct (Radostits *et al.*, 2000) and begins with the ingestion of the sporulated oocysts by the susceptible host. Following ingestion by suitable host, the oocysts undergo excystation and release four infective sporozoites, which exit from a suture located along one side of the oocyst. Each sporozoite invade the microvillous brush border of the enterocytes and differentiates into a spherical parasite, called trophozoite, which in turn multiplies asexually to form two types of meronts (formerly called schizonts), each about 5µm in diameter. This meronts are termed Type 1 meronts which produce six to eight new banana-shaped parasites (merozoites). Some Type I merozoites are somehow triggered into forming a second type of meront, Type 2 meronts, which form four oval-shaped merozoites, called the gametocytes. The gametocytes invade new intestinal cells, where they differentiate into male cells (microgametocytes) and female cells (macro-gametocytes) (O´Donoghue, 1995). Then these microgametocytes (males) and macro-gametocytes (females) produce zygote by fertilization, which undergoes further development into an oocyst. The oocysts may be either thick- or thin-walled oocysts. Thin-walled oocysts may excyst within the same host and cause autoinfection, while thick-walled oocysts are excreted with the faeces and are infective to the new host (Fig.1). The parasite begins its life cycle as new when these oocysts are ingested by a new host. The infection spread to new hosts by a subsequent shedding (Holland, 1990; Radostits *et al*., 2000).

3.4. Pathogenesis

Cryptosporidium affects immunosuppressive animals either as primary pathogens or secondary invaders. *Cryptosporidium parvum* causes acute infectious diarrhea in humans and animals. The underlying mechanisms by which *Cryptosporidium* causes diarrhea, malabsorption and wasting are complex and still not fully understood (Gookin, 2002).

Cryptosporidium does not infect tissue beyond the superficial surface epithelia. The organism remains just beneath the luminal cell membrane of the intestinal epithelial mucosa and damages it either through a direct parasitic invasion, multiplication, and extrusion or through T cell-mediated inflammation. The damage results in villous atrophy, crypt hyperplasia, and infiltration of lymphocytes, neutrophils, plasma cells and macrophages into the lamina propria. Due to this damage to the epithelial mucosa cells, it releases cytokines that activate resident phagocytes. These activated cells release soluble factors (interferon gamma, prostaglandins, histamine, adenosine, and serotonin) that increase intestinal permeability and secretion of chloride and also inhibit absorption (Goodgame, 2003).

3.5. Clinical signs

Variation in symptoms may represent and used as additional indicators to set up specific diagnosis for *Cryptosporidium* detection and bring about correlation between infecting species and epidemiology. The severity and persistence of the clinical sign is dependent on parasite characteristics, infective dose, current infection with other pathogens such as rotaviruses and host factors. Host factors depend on immune status and frequency of exposure of the infected individual (Meinhardt, 1996). Clinical signs are essentially frequent, watery diarrhea which occurs between the ages of 5 and 20 days. The severity is moderate to high in calves and very high in kids (Fayer, 1998).

3.6. Diagnosis of *Cryptosporidium*

There are many diagnostic tests for *Cryptosporidium* including microscopy, staining, and detection of antibodies. The symptoms of cryptosporidiosis are not pathognomonic. Because of the small size of the *Cryptosporidium* oocysts, they are difficult to identify in fresh samples without specific coloration and laboratory verification to confirm the diagnosis. This is usually done by the detection of oocysts in faeces after concentration by different techniques and microscopic examination of smears stained with different staining method (Urquhart *et al.*, 1996; Radostits *et al.,* 2000).

Oocysts may be concentrated by different technique such as, modified zinc sulfate centrifugal flotation technique or by Sheather's sugar flotation, formalin-ethyl acetate sedimentation followed by layering and flotation over hypertonic sodium chloride solution to separate oocysts from stool debris (Weber *et al*., 1991). After concentration by different techniques, oocysts may be examined under phase-contrast microscopy and appears as spherical bodies. After concentration, several staining techniques can be used. One staining method is the Modified Ziehl-Neelsen technique. It is the best staining technique, which stains granules of the sporozoites with a bright red colour and blue or green background depending on the counter stain used. The oocysts appear as rose spherical elements (Weber *et al*., 1991; Sunnotel *et al*, 2006).

Oocysts can also be detected by direct immunofluorescent assays that are commercially available utilizing monoclonal antibodies raised to *Cryptosporidium* antigens. For this type of techniques there are a variety of commercial kits are available. Immunofluorescence-based kits, using a fluorescein isothiocyanate-conjugated anti-*Cryptosporidium* MAb that recognizes surface exposed epitopes of oocysts (FITC-*C*-MAbs) are more specific for, and can be more sensitive at, detecting *Cryptosporidium* oocysts in faecal smears. Immunofluorescent stains are more specific (Jex R., 2008).

Alternatively, oocyst antigens capture methods like, enzyme linked immunosorbent assays (ELISA) is one of the recent detection method of oocysts made by the use of copro-antigen detection kits. Depending on the commercial kit, *Cryptosporidium* coproantigen (cell wall proteins) are captured and developed using a mixture of monoclonal and polyclonal antibodies. Enzyme linked immunosorbent assay (ELISA) is used to demonstrate the endogenous parasite stages attached to the brush border of epithelial cells (Johnston *et al*., 2003).

3.8. Treatment

There is no effective or approved treatment for Cryptosporidiosis. The role of antiparasitic therapy is not significant for the treatment of cryptosporidial infection. The resistance to treatment results from the parasite location separated from the lumen by the host membrane but also segregated from the host cytoplasm by the base of the parasitophorous vacuole. Thus, there is limited exposure to drugs in the lumen, serum, and even in the enterocyte cytoplasm. Since cryptosporidiosis is a self-limiting illness in immunocompetent individuals, general, supportive care is the only treatment for the illness (Chen, 2002). Oral rehydration solution, containing glucose, sodium bicarbonate, and potassium, intravenous rehydration and replacement of electrolytes may be necessary for particularly voluminous, watery diarrhea. Passive oral transfer of protective antibody in hyperimmune bovine colostrum (HBC) has also led to marked improvement in symptoms in some immunocompromised patients and in animals with severe cryptosporidiosis, but antibody preparations have not become clinically available (Griffiths, 1998).

For immunocompromised patients with cryptosporidiosis, antibiotics such as spiramycin and diclalzuril sodium are the possible treatments for the illness. They produce partial decrease in number of oocyst and diarrhea, but have not yielded reliable result. However, one particular antimicrobial agent, paromomycin (a nonabsorbable aminoglycoside) is an effective antibiotic which decrease the intensity of the disease and improve intestinal function. The treatment efficiency of paromomycin is increased when it is combined with serine protease inhibitors. In addition Nitazoxanide is also used as an important treatment, which significantly decreases the duration of *C. parvum*-associated diarrhea and oocysts shedding (Rossignol, 2001).

3.7. Prevention and Control

Because all *Cryptosporidium* infections are initiated through ingestion of oocyst, control of this stage is the most important method in limiting the spread of the disease (Chen, 2002). Elimination of the parasite is impossible, because infected animals and humans will continue to contaminate the environment. The control of *Cryptosporidium* can only be achieved by combining a good hygiene management and effective preventive drugs. Filtration is also particularly important when surface contamination may occur in water sources. It is capable of removing particles less than 1 μm in diameter. At-risk persons should avoid contact with obvious sources of *Cryptosporidium* oocysts, such as people with diarrhea, farm animals (particularly cattle), and domestic pets that are either very young (< 6 months), have diarrhea, or have been stray (Kaplan, 2002).

Infection control measures are held by a variety of commercial disinfectants, most of these have little or no effect on parasite infectivity even when *C. parvum* oocysts were exposed at intervals ranging from 30 minute to 24 hour. Disinfection with chlorine has always been an important barrier for waterborne pathogens, but *Cryptosporidium* oocysts are still resistance against chlorine disinfectant. For effective prevention exposure of *Cryptosporidium* oocysts to multiple disinfectants is better than using a single disinfectant alone. Prolonged exposures to gaseous or aqueous solutions of ammonia, hydrogen peroxide, high concentrations of chlorine dioxide and related compounds, and short term exposure to ozone are slightly more effective in reducing *cryptosporidium* oocyst. Ozone is the most effective chemical disinfectants that able to kill oocysts at higher temperature (Finch *et al*., 1994; Liyanage *et al*., 1997).

Various physical stresses such as heat, cold, radiation, pressure, and desiccation are the most effective and economical method of reducing the numbers of oocysts in the environment. They do not survive for a long time, if the temperature decreased below 5°C or increased above 15°C. Exposure of oocysts at different temperatures affects their carbohydrate energy reserves stored in the sporozoites, and the residual bodies such as amylopectin granules, which serve as the excystation process and host-cell invasion are consumed more rapidly at higher temperatures (Jenkins *et al*., 2003).

3.9. Public health importance of *Cryptosporidium*

Cryptosporidium parvum is the most important zoonotic agent of cryptosporidiosis, with a large range and abundance of animal reservoirs, mainly in young farmed animals. It is the major causes of diarrheal diseases in humans worldwide and is included in the World Health Organization's Neglected Disease Initiative. In addition to the occurrence of diarrhea, cryptosporidiosis has been attributed to malnutrition and stunted growth. The Centers for Disease Control (CDC) has also documented the importance of *C. parvum* as a major human pathogen. Outbreaks of cryptosporidiosis have been reported among veterinarians and veterinary students, other people exposed to agricultural animals and children visiting farms (Savioli *et al*., 2006).

Infections of the human gastrointestinal tract with enteric pathogens are among the leading causes of disease, suffering, and death worldwide. This enteric infection is caused mainly by the zoonotic species *Cryptosporidium parvum,* and *Cryptosporidium hominis* which is highly prevalent in contaminated water and food. Prolonged diarrheas in early childhood are often associated with poor mental function, failure to thrive and increased risk of stunting (Putignani and Menichella, 2010).

Peoples in developing countries are more vulnerable to persistent infection, because the infection is dependent on malnutrition, HIV infection and strength of immune system. This has been related to impaired physical fitness in late childhood. In developed countries, cryptosporidiosis also occurs in adults due to food borne or waterborne outbreaks. About 20% of all cases of childhood diarrhea are results from *cryptosporidium* infection and it is also a potentially fatal complication of AIDS (Mosier and Oberst, 2000).

3.10. Economic importance of *Cryptosporidium*

Cryptosporidium is considered an important food borne pathogen causing a disease of socioeconomic significance worldwide. Factors occurred by Cryptosporidiosis in animals include increased environmental contamination and trends in livestock production. Subclinical cryptosporidiosis may result in reduced body weight, poor growth rates in calves and milk production loss in adult dairy cattle. Despite improvements in the health status of livestock in commercial units, cryptosporidiosis is still associated with significant morbidity and mortality (Anderson, 1988).

The cost expended for litigations and infrastructure improvements in water treatment facilities during the outbreak is very high. It also needs a legal requirement for removal of *Cryptosporidium* from drinking water supplies. This requires a large amount of budget for researchers and professionals to deal on the disease, which may decline the country's economy (Corso, 2003).

4. Conclusion And Recommendations

Cryptosporidium infection is one of the most important protozoal diseases with high morbidity and mortality rates in calves. Even though the severity is high in neonates, the disease affects all age groups. This helps the parasite to have a wide range of host including those animals that have close relation to humans and then zoonotic transmission is possible. The disease occurrence, source of infection, host range, and morphology makes it difficult to prevent or needs much effort to find appropriate drug for permanent treatment. *Cryptosporidium* is capable of completing all stages of its development within a single host and cause immune compression up to mortality.

Therefore based on the above conclusion the following recommendations are forwarded:

- Keeping good hygiene and sanitation to avoid contamination.
- Emphasis should be given to the health of calves.
- Avoid consumptions of contaminated water and foods in order reduce the risk of exposure.

Acknowledgements

An above all praise and heartily thanks to God the almighty creator lord of the universe for this innumerable favours and benevolence up on us through all walks of our life and keep our healthy. Secondly we would like to express our grateful thanks and sincere to our advisor Dr. Basaznew Bogale for the guidance, whole hearted advice and provision of literature material and devotion of incalculable time correction of this seminar paper.

References

1. Addiss, D.G., Pond, R.S. and Remshak, M., 1993. Reduction of risk of watery diarrhea with point of use water filters during a massive outbreak of waterborne cryptosporidium infection in Milwaukee, Wisconsin; 54, p.549-553.
2. Anderson, C., 1988. Gastric cryptosporidiosis of feeder cattle, beef cows and dairy cows: *Bovine Practitioner* 23, p. 99-101.
3. Anderson, B., 1998. Cryptosporidiosis in bovine and human health. *Journal of Dairy Sci*ence, 81, p.3036-3041.
4. Barriga, O.O., 1997. Veterinary parasitology for practitioners, 2nd ed. Edina: Burgess International Group.
5. Caccio, S.M., Thompson, R.C. and McLauchlin, J., 2005. Unraveling *Cryptosporidium* and *Giardia* epidemiology. *Trends Parasitology,* 21(9), p.430–437.
6. Casemore, D.P., Wright, S.E, Coop, R.L., 1997. Cryptosporidiosis – Human and animal epidemiology. In: Fayer R (ed). *Cryptosporidium and* cryptosporidiosis. CRC Press, Boca Raton, USA, p. 65-92.
7. Chen M., Keithly, S., Paya, V. and LaRusso, F., 2002. Cryptosporidiosis. *England Journal of Medicine*, 346(22), p.1723–1731.
8. Corso, P. S., M. H. Kramer, K. A. Blair, D. G. Addiss, J. P. Davis and A. C. Haddix., 2003. Cost of illness in the 1993 waterborne *Cryptosporidium* outbreak, Milwaukee, Wisconsin. *Emerging Infectious Disease*, 9, p. 426–431.
9. Current, W., 1994. *Cryptosporidium parvum* household transmission. *Annals Internal Medicine,* 120, p.518-519.
10. Desai, N.T., Sarkar, R. and Kang, G., 2012. Cryptosporidiosis: An under-recognized public health problem. *Tropical Parasitology,* 2, p. 91-98.

11. Fayer, R., Gasbarre, L. and Pasquali, P., 1998. Cryptosporidium parvum infection in bovine neonates: dynamic clinical, parasitic and immunologic patterns. *International Journal for Parasitol*ogy, 28, p. 49-56.
12. Finch, G., Kathleen, B. and Gyurek, L., 1994. Ozone and chlorine inactivation of *Cryptosporidium.* Preceding of American Water Works Association Water Quality Technology Conference, November 1994, San Francisco, American Water Works Association, Denver, USA.
13. Flanigan, T.P. and Soave, R., 1993. Cryptosporidiosis. *Progress in Clinical Parasitology*, p.1-20.
14. Goodgame, R.W., 2003. Understanding intestinal spore-forming protozoa: cryptosporidia, microsporidia, isospora, and cyclospora. *Annals Internal Medicine*, 124 (4), p. 429-441.
15. Gookin, L., Nordone, S. and Argenzio, R., 2002. Host response to cryptosporidium infection. *Journal of Veterinary Internal Medicine,* 16, p.12-21.
16. Griffiths, K., Balakrishnan, R., Widmer, G. and Tzipori, S., 1998. Paromomycin and geneticin inhibit intracellular *Cryptosporidium parvum* without trafficking through the host cell cytoplasm: implications for drug delivery. *Infection and Immunity*, 66, p.3874-3883.
17. Hoard's Dairyman, 2012. Cryptosporidium parvum is silently stealing pounds and threatening your family. [Online]. Available at: (http://www.hoards.com/IB_MGB-C-parvum) [accessed on10 April 2016].
18. Holland, R.E., 1990. Some infectious causes of diarrhoea in young farm animals. *Clinical Microbiology Review* 3, p. 345-375.
19. Jenkins, M., Trout, J.M., Higgins, J., Dorsch, M., Veal, D. and Fayer, R., 2003. Comparison of tests for viable and infectious Cryptosporidium parvum oocysts. *Parasitology Research,* 89, p. 1-5.
20. Jex, R., Smith, H., Monis, P., Campbell, B. and Gasser R., 2008. *Cryptosporidium* biotechnological advances in the detection, diagnosis and analysis of genetic variation. Biotechnology Advances, 26, p. 304–317.
21. Johnston, S.P., Ballard, M.M., Beach, M.J., Causer, L. and Wilkins, P.P., 2003. Evaluation of three commercial assays for fecal specimens. *Journal of Clinical Microbiology*, 41, No.2, p. 623-626.
22. Kaplan, J., Benson, C., Holmes, K., Brooks, J., Pau, A. and Masur, H., 2009. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *The Morbidity and Mortality Weekly Report Recommendations and Reports,* 58, p.1–207.
23. Karanis, P., Kourenti, C. and Smith, H., 2007. Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. *Journal of Water and Health,* 5(1), p.1–38.
24. Keusch, G.T., Hamer, D., Joe, A., Kelley, M., Griffiths, J. and Ward, H., 1995. "Cryptosporidia—who is at risk?" *Schweizerische Medizinische Wochenschrift*, 125 (18), p. 899-908.
25. Lendner, M., Etzold, M., Daugschies, A., 2011. Cryptosporidiosis-an update. *Berliner und Münchener tierärztliche Wochenschrift,* 124, p. 473-484.
26. Liyanage, R.J., Finch, G.R. and Belosevic, M., 1997. Sequential disinfection of *Cryptosporidium parvum* by ozone and chlorine dioxide. *Ozone Science and Engineering,* 19, p. 409-423.
27. Meinhardt, P.L., Casemore, D.P. and Miller, K.B., 1996. Epidemiologic aspects of human cryptosporidiosis and the role of waterborne transmission. *Epidemiologic Review,* 18, p.118–136.
28. Mosier, D. and Oberst, R., 2000. Cryptosporidiosis: A global challenge. *Annals of the New York Academy of Sciences*, 916, p.102-111.
29. O'Donoghue, P.J., 1995. *Cryptosporidium* and cryptosporidiosis in man and animals. *International Journal Parasitology*, 25(2), p.139–195.
30. Pandak, N., Zeljka, K. and Cvitkovic, A. A., 2006. Family outbreak of cryptosporidiosis: probable nosocomial infection and person-to-person transmission. *Wiener Klinische Wochenschrift*, 118, p. 485–710.
31. Putignani, L. and Menichella, D., 2010. Global distribution, public health and clinical impact of the protozoan pathogen *cryptosporidium. Interdisciplinary Perspectives on Infectious Diseases*.
32. Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchcliff, K.W., 2000. Veterinary Medicine, 9 ed: W.B Saunders. 137-144, p.1310-1314.
33. Rossignol, F., Ayoub, A. and Ayers, M., 2001. Treatment of diarrhea caused by *Cryptosporidium parvum*: a prospective randomized, double-blind, placebo-controlled study of Nitazoxanide. *Journal of Infectious Disease*, 184, p.103-106.

34. Savioli, L., Smith, H. and Thompson, A., 2006. *Giardia* and *Cryptosporidium* join the 'Neglected Diseases Initiative'. *Trends Parasitology,* 22, p.203-208.
35. Scallan, E., Hoekstra, R., Angulo, F., Tauxe, R., Widdowson, M., Roy, S., Jones, J. and Griffin, P., 2011. Foodborne illness acquired in the United States--major pathogens. *Emerging Infectious Diseases*, 17(1), p.7-15.
36. Singh, B.B., Sharma, R., Kumar, H., Banga, H.S., Aulakh, R.S., Gill, J.P.S. and Sharma, J.K., 2006. Prevalence of *cryptosporidium parvum* infection in Punjab (India) and its association with diarrhea in neonatal dairy calves. *Veterinary parasitology,* 140, p. 162-165.
37. Sunnotel, O., Lowery, C., Moore, J., Dooley, J., Xiao, L., Millar, B., Rooney, P. and Snelling, W., 2006. *Cryptosporidium*. *Letters Applied Microbiology*, Vol.43, No.1, p. 7 16.
38. Tzipori, S., M. Smith, C. Birch, G. Bames and R. Bishop, 1983. Cryptosporidiosis in hospital patients with gastroenteritis. *The American Journal of Tropical Medicine Hygiene,* 32, p.931-934.
39. Urquhart, G., Armour, J., Duncan, J., Dunn, A. and Jennings, F., 1996. Veterinary parasitology, 2 ed. UK: Blackwell science. P. 233-234.
40. Weber, R., Bryan, R., Bishop, H., Wahlquist, S., Sullivan, J. and Juranek, D., 1991. Threshold of detection of *Cryptosporidium* oocysts in human stool specimens: evidence for low sensitivity of current methods. *Journal of Clinical Microbiology*, 29, No.7, p.1323-1327.
41. Xiao, L., 2000. Identification of species and sources of Cryptosporidium oocysts in storm waters with a small-subunit rRNA-based diagnostic and genotyping tool. *Applied Environmental Microbiology*, 66, p.5492–5498.
42. Xiao, L., Fayer, R., Ryan, U. and Steve, J., 2004. *Cryptosporidium* Taxonomy: Recent Advances and Implications for Public Health. *Clinical Microbial Reviews*, 17, No.1, p.72-97.

1/25/2017