A Review On Cryptosporidiosis In Calves

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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.

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.
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

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

Source: Xiao et al., 2004

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 transformed 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 (macrogametocytes) (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 signs 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 diclozuril 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 amyllopectin 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.

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