



REVIEW ARTICLE

Risk Factors Analysis of Bovine Cryptosporidiosis in Diarrheic Calves

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ABSTRACT

Cryptosporidiosis is a significant enteric disease in cattle, particularly affecting young calves and contributing to severe diarrhea, morbidity, and mortality. This study aims to analyze the risk factors associated with bovine cryptosporidiosis in diarrheic calves. Risk factors, including environmental conditions, calf age, management practices, and herd size, were assessed through structured questionnaires and farm visits. Multivariate logistic regression was employed to determine the significance of each factor. The results indicated that calves under age weeks, housed in poor hygiene conditions, and raised in larger herds had a higher prevalence of cryptosporidiosis. Other significant factors included inadequate colostrum intake, mixed infections, and seasonal variations. These findings highlight the need for improved management practices, early diagnosis, and targeted interventions to reduce the burden of cryptosporidiosis in calf populations.

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1. Introduction

Cryptosporidium spp are apicomplexan parasite causing infections in wide range of hosts including higher and lower animals. Approximately 152 mammalian hosts have been described as a source of infection and the parasite lives in their large and small intestines and respiratory tracts of small and large ruminants [1]. *Cryptosporidium* currently consists of 23 species and over 40 genotypes, and new genotypes are constantly being discovered [1,2]. Fayer, [2], reported based on recent molecular studies, it has been determined that cattle have minimum five type of *Cryptosporidium* parasites; *Cryptosporidium parvum*, *Cryptosporidium bovis*, *Cryptosporidium andersoni* and *Cryptosporidium ryanae*, and *Cryptosporidium suis*. of Molecular approaches revealed that the cattle are infected by at least sixteen species and genotypes of *Cryptosporidium*. According to Moore and Zeman [3], among them *Cryptosporidium parvum* is one of the most frequently detected pathogenic organisms in calves under

the age of three weeks. Additionally, *Cryptosporidium parvum* is an important zoonotic species since it is involved in the majority of human cases of cryptosporidiosis [2]. In children, *Cryptosporidium* causes diarrhea due to foodborne or waterborne outbreaks [4]. In healthy adults, symptoms of cryptosporidiosis may range from mild to severe and last for up to two weeks [5], on the other hand, in immunocompromised individuals, cryptosporidiosis may manifest as chronic or extra intestinal infections [6], as well as the treatment required; administration of electrolytic solutions, drugs, and appropriate hygiene practices. It has been estimated that infected calves in Great Britain cost between \$100 to \$200 per animal as reported in 2014 [7], mostly due to veterinary treatment, a reduction in milk yield, and a lower weight gain.

A large number of cattle are infected with *C. parvum* [8]. Cattle may serve as reservoirs for *Cryptosporidium* species that cause cryptosporidiosis in humans. A young calf (less than six weeks old) is particularly susceptible to

infection. In Various European countries 20%-40% cryptosporidiosis has been reported in young calves young calves. There is a higher prevalence of cryptosporidiosis in early age of calves between the 1-2 months while younger calves than one month of age. Kahar *et al* [9], reported 28.18% cryptosporidiosis in two selected areas of Bangladesh. There is a higher prevalence of cryptosporidiosis among men (34.75%) than among women (24.64%). *Cryptosporidium* subtypes that are zoonotic are more likely to be present in calves aged 15–21 days, [10], suggest that both diarrheic animals and young children are at risk for the transmission of cryptosporidiosis. In a study conducted by (Khar *et al* 2014), *Cryptosporidium* species were found to be prevalent in calves that were pre-weaned and post-weaned. 35 % prevalence of *Cryptosporidium* was followed in post-weaned calves 30 % by *C-andersoni* and *C-ryanae* and 5% mixed respectively while in pre-weaned calves was followed by 32 % by *C-Parvum*, 26% *C-Bovis*, 20% *C-andersoni*, 11% *C-ryanae* and 8% mixed species respectively.

2. Prevalence of the *Cryptosporidium* infection in large ruminants

A study was conducted by [11], reported different pathogens caused Non communicable diseases(NCDs) and mortality rate in dairy calves at Uruguayan and whether these infections, diarrheas, and deaths were associated with failure to transfer passive immunity (FTPI). An analysis of 27 different farms under the age of 1 to 30 day-old diarrheic and non-diarrheic calves was conducted.

ELISA was confirmed that Bovine retrovirus, coronavirus and E-coli were detected antigen, while PCR was detected bovine astovirus and finally RT-PCR was detected salmonella species. Bovine viral diarrhea virus (BVDV) was also detected by RT-PCR or antigen-capture ELISA on blood and serum samples. Refractometer was used to determine the concentration of serum total proteins (STP) in the serum of 95- 8-day-old calves (n=95). The number of calves that died prior to weaning among the sampled calves. In 65.4% of the calves, At least one pathogen was detected in 65% of calves and this percentage was significantly higher in diarrheic calves (83.7%) than non-diarrheic calves (47.6%). Compared to other pathogens, *Cryptosporidium* spp and Rotavirus were associated with NCDs. *Salmonella enterica*-infected diarrheal calves were at an increased risk of death. A decrease in calf morbidity and mortality could be achieved in Uruguay if NCDs, salmonellosis, cryptosporidiosis, and rotavirus infections were controlled and colostrum management practices were improved. Khan *et al.* [12], investigated the molecular prevalence of *Cryptosporidium* in district Nowshera, Pakistan. A total of 300 water samples were collected and analyzed using PCR. A total of 30.33% numbers of the samples tested positive for infection, with the infection rate being higher in drain water and lower in tube well water.

A total of 50 diarrheic calves were screened for cryptosporidiosis on various farms in and around Bidar by [14]. A total of six calves between the ages of six days and six months were examined. The prevalence of 4% was determined by direct microscopic examination under (100x) oil immersion. Modified zheil Neelsen acid fast and safranin staining techniques revealed prevalence rates of 18% and 16%, respectively. The effect of *Cryptosporidium* was detected in fecal samples by PCR, which resulted in 43.33% positivity in 30 fecal samples. In a study of 13 samples, 7 were found to be positive for *Cryptosporidium parvum* by immuno-chromatography test-based kits (DipFitTM, BioX Diagnostics, Belgium).

A study was conducted in Peninsular Malaysia to check the prevalence of *Cryptosporidium* on dairy cattle feces samples (824 different sample and 28 different farm) and beef samples (526 sample in 298 different farm) and used PCR tool make different specific primers for identification the targeted genes. Among cattle, *Cryptosporidium* infection was prevalent at 12.5%, with significant breed-specific differences. There was no significant difference the rate was observed between the genders (male and female) or breeds (beef and dairy cattle) [15]. Diaz *et al.*, [16], conducted microscopic examination of 147 fecal samples, that were collected from 22 different herds. All the positive samples were subjected to molecular confirmation. *Cryptosporidium parvum* (95.8%) and *Cryptosporidium bovis* (4.2%), that were identified by PCR amplification of the SSU rRNA gene. Khushdil *et al.* [16], also examined 52 children's fecal samples with diarrhea. Where 20.3% samples were detected positive for *Cryptosporidium* infection. It was concluded that *Cryptosporidium* is a common parasite persistent in diarrheal patients throughout the country.

A study was conducted by Shafiq *et al*, [17], who recorded *Cryptosporidium* infections in small ruminants and their transmission through water. He collected feces samples from 300 sheep and while water samples were collected from various locations. He observed 28% oocysts were in canal water, 85% in fresh water and 4% below earth water. A prevalence of 18.66% was reported in sheep, while a prevalence of 21.33% was reported in goats. A protozoan parasite, *Cryptosporidium*, can cause some intestinal issues in vertebrates including humans. The present study showed that calves is the major sources for prevalence the *Cryptosporidium*. Globally, there are 119 different dairy and beef cattle sample were collected from 10 different farms in Parish, where the prevalence of the disease is estimated to be about 21 percent [18]. The Polymerase Chain Reaction (PCR), which amplifies the 18S rRNA gene, confirmed that *Cryptosporidium* was present in 10 (8 %) of the samples examined. A study was conducted by Alhaji *et al.* [19], to determine the prevalence of *Cryptosporidium* in cattle feces in Nigeria by examining the oocysts. The MZN staining technique was used to detect

Cryptosporidium oocysts in a total of 400 cattle fecal samples. He observed 17.8% prevalence of *Cryptosporidium* infection. As a result, the prevalence of *Cryptosporidium* infection in cattle was highest in Gwagwalada Area Council (32.4%, 22/68). Similarly, study was also conducted in Lahore Pakistan where cryptosporidiosis was highly prevalence in diarrheal calves. A total of 500 faecal samples were collected from buffalo calves, 250 from cow calves, and 250 from cow and buffalo calves, respectively, from different parts of the world and from different dairy forms and then analysed using the Acid Fast staining technique. They found that overall, 25.6% of calves shed *C. parvum*, with prevalence's of 27.2% and 24% in cows and buffaloes, respectively. It was found that the pattern of oocyst shedding was negatively related to age, with the highest level at the age of 1-30 days and while the lowest level in the 9-month to one-year of age group. It was observed that both shading and shading intensity were significantly higher ($P<0.05$) in diarrheal calves than in non-diarrheal calves. A significant association has been found between *C. parvum* and diarrhoea in dairy calves in Lahore, based on the findings of this study [20].

The *Cryptosporidium* parasite causes epidemics of cryptosporidiosis around the world where it is found in lakes and watercourses polluted with sewage and animal waste. It is the most common cause of waterborne zoonotic illnesses in the world. According to [21], collected 300 water samples from four different flood-affected districts. These samples were used to determine the prevalence of *Cryptosporidium* by using polymerase chain reaction (PCR). There was an overall prevalence of 30.33% (91/300) for *Cryptosporidium*, with a higher prevalence of 44% in drain water and a lower prevalence of 5% in bore/tube well water. A prevalence of 33% was observed in open well water, while a prevalence of 20% was observed in tap water.

3. Historical point of view

In 1885, a scientist named Clark reported first parasite in mice epithelium organs which resemble with *Cryptosporidium* and late of 1907 was experimentally proved by American parasitologist E, Tyzzer that the protozoa found in laboratory mice species not in wild mice.

It was not until 1910 that he provided characteristics for the establishment of *Cryptosporidium muris* as a new genus. The characteristics of the *Cryptosporidium* such as reproduction, development was observed in *C. Muris* and the spore are released from the gland of mature oocyte. Fayer & Ungar, [22], *Cryptosporidium* was first discovered, and over half of these years have been spent mistaking it for other apicomplexan genera, especially *Sarcocystis*, a coccidian species. As a result of the thin walls of many

Sarcocystis species oocysts, which often rupture, releasing free sporocysts, as well as the fact that each sporocyst contains four sporozoites, many species of this genus have been incorrectly assigned to it [23].

The parasite *Cryptosporidium Parvum* causes cryptosporidiosis, a parasitic infection. Once infected by the protozoan parasite, Crypto. This parasite is present in the intestines and is passed in the stool of the individual suffering from the infection. Diarrhea, dehydration, cramps, and nausea are common symptoms of diarrheal infection. As a result of its prevalence in the United States during the past two decades, the Crypto virus has gained a great deal of notoriety. The disease is spread through contaminated food and water. In order to prevent and control this disease, it is very important to maintain cleanliness. The first time disease was observed causing from the *Cryptosporidium* in 1976 in the human's faeces samples. Initially, Crypto was considered a veterinary problem due to the fact that it was most commonly detected in handlers of dairy farm animals such as cows. It has been reported that 155 mammal species are infected with *Cryptosporidium parvum* [24]. Edward Ernst Tyzzer [25], discovered the genus *Cryptosporidium* in the gastric glands of mice in 1910. The oocysts and sporulates of this parasite are not accompanied by sporocysts during attachment to the host, according to Tyzzer. Accordingly, Tyzzer referred to the parasite known as *Cryptosporidium muris* as a member of the genus *Cryptosporidium*, derived from the Greek word "kruptos," meaning "hidden." Three years later, Tyzzer identified a third species of *Cryptosporidium* that was not infectious in mice's stomachs or small intestines. It was not until 1961 that the first case of cryptosporidiosis on an animal was recorded due to Tyzzer's discovery of new parasites. Further, a three-year-old girl who presented with abdominal pain and diarrhea also presented with these symptoms [27]. This suggests that synanthropic dirt flies may be a potential source of transmission for human cryptosporidiosis.

4. Taxonomic Classification of *Cryptosporidium*

A lot of parasite can cause a series of threats to human beings and animals like *Cryptosporidium* is one of the parasite that infect the animal's gastrointestinal tract such as most disease is diarrheic and mostly susceptible in more than two years' children in developing country [28]. *Cryptosporidium* is classified in the Apicomplexa phylum, Sporozoasida class, Coccidiasina subclass, Eucoccidiida order, Eimeriina suborder, family Cryptosporidiidae family. The life cycle of *Cryptosporidium* was similar to the *Eucoccidiida* coccidian parasites and mostly found in gastrointestinal tract [29], and a number of genera are monoxenous in their life cycles, taking place in the same host species throughout each stage of parasite development.

Totally 22 different type of *Cryptosporidium* species are reported which 13 species were confirmed in humans and domestic animals where *Cryptosporidium parvum* is the main cause of disease [29].

5. Morphology of *Cryptosporidium* Oocyst

In different stages of the parasite, *Cryptosporidium* has different morphological characteristics. The oocysts released into the surroundings are ovular in form with a smooth surface. In addition to its thick wall that makes it resistant, it also has a cleft on one side that allows the release of sporozoites during excystation. The size of these oocysts is approximately 5 by 7 microns. Sporozoites measure approximately 5x0.5um and are characterized by a rough surface, a rounded apical region, and a rough surface. In contrast, trophozoites have a smooth surface and a hood like shape and length is between 1 to 15 mm measured. The size of Type I and Type II meronts is different (1.5 mm and 3.5 mm respectively), but they both have an epicellular, smooth surface and their merozoites are of similar size (0.4 mm and 1 mm). A rod-like structure is characteristic of type I merozoites with a pointed apical region and rough surface, while a round shape and rough surface characterize type II merozoites. The microgametes formed from Type II merozoites measure approximately 0.1um with a spherical and rough surface, whereas the macrogametes measure 4x5um with an ovular and rough surface [31].

6. Life cycle of *Cryptosporidium*

Robertson *et al.* [32], were reported that *Cryptosporidium oocyte* are ingested to the host through any sources then cause disease because the spore is release inside the host digestive tract and formed a colony. Sporozoites leakage from the oocyst through a slit-like opening when dissolve the special structure of oocyte [33]. After dissolve then the oocyte free from the epithelial cell, they become condensed within the vacuoles and attached to other organelle called, trophozoites and start asexual proliferation (formerly known as schizogony). As a result of endopolygeny formed in the mother cell by internal budding. Mostly two types *Cryptosporidium* meronts are present [34]. The type 1 consist of eight merozoites, which are released from the parasitophorus vacuole after maturity. The merozoites start the attacking to epithelial cells where they undergo transformation into type II meronts. Type II meronts produce four merozoites that do not undergo further reproduction, and undergo sexual reproduction and formed microgamonts (males) and macrogamonts (females) [35]. The microgametocytes produce 16 non-flagellated microgametes. In the presence of mature microgametes, macrogamonts develop into uninucleate macrogametocytes. On the other hand, sporogioform produced by asexually with four sporozoites are formed. The majority 82 % of thick-walled oocyte

produced and excreted from the host in faeces. It has been found that a small percentage of oocysts (20%) are thin-walled and may excyst within the same host animal thereby causing a new cycle of infection (autoinfection). Such auto-infections are thought to provide hosts with persistent chronic infections [36].

7. Developmental stages Period/ Clinical Presentation

Cryptosporidium species and genotypes can cause a wide range of symptoms. An average incubation period of seven days is observed (range: two to ten days). Immuno competent patients may experience diarrheal that is self-limiting and resolves within two to three weeks. It is possible for immunocompromised patients more severe difficulties such as protein deficiency disease, weight losses, diarrheic disease and fatigue. Although cryptosporidiosis is primarily associated with the small intestine, extra intestinal cryptosporidiosis has been reported.

8. Mode of transmission and source of infection

The transmission mode of *Cryptosporidium* occurred when the healthy species ingested oocyst from the faeces of infected persons or animals. As a result, cryptosporidial infections can be transmitted between people, through ingesting fecally contaminated food or water, from animal to animal, or through being in direct contact with feces or feces-contaminated surfaces. Poor faecal-oral hygiene is typical of the transmission of cryptosporidiosis and giardiasis, caused when contaminated materials are swallowed by hosts who are susceptible to infectious oocysts contained in feces. Among the most common means of transmission are water and food, although direct contact between people or animals is also an important route for transmission. A person with an immune compromise (for example, a person with AIDS or cancer, a young or elderly person, etc.) is most likely to suffer from the consequences of these diseases. In 2004, the World Health Organization included *Cryptosporidium* and Giardia infections in the Neglected Disease Initiative because they pose a considerable socio-economic burden in developing countries.

9. Transmission via Water and Food

Over 400,000 people in Milwaukee were affected by a cryptosporidiosis outbreak in 1993, which was attributed to drinking water [37]. Water from lakes, rivers, swimming pools and streams was used by utility companies in outbreaks, as well as water from wells and springs [37]. *Cryptosporidium* species may be transmitted through drinking water at low levels (nonepidemic) throughout the United States, according to circumstantial evidence. Researchers found *Cryptosporidium* oocysts in 65%-97% of surface water samples across the country [38].

10. Direct transmission between humans

Typically, *Cryptosporidium* and Giardia spread from person to person, as a result of poor hygiene and sanitation. Day-care centres are a common source of cryptosporidiosis and giardiasis infection, with diaper alterations and mouthing behaviour being the most common causes of infection. Both patient-to-patient and patient-to-health care worker transmissions of these diseases have also been reported in nosocomial settings. Moreover, *Cryptosporidium* has been detected in both sputum and vomitus, suggesting that these body fluids also help to transmission. Sexual relation is also one the *Cryptosporidium* transmission factor [38].

11. Transmission between animals and humans

All over the world mammalian, avian reptiles are reported to *Cryptosporidium* [39]. Several studies have demonstrated that *C. Parvum* are lesser extent than zoonotic. *C. parvum* is primarily transmitted to humans through new-born calves, whereas *C. andersoni* is transmitted through older animals. Among other methods of transmission, direct contact with infected young cattle or consumption of livestock-contaminated water can lead to transmission. At least one waterborne outbreak has been linked directly to cattle [33]. Sheep and goats are less likely to be infected with *C. Parvum* than lambs to children and handlers [40]. Sheep and goat are infected from *Cryptosporidium* species is limited evidence to support this hypothesis. *Cryptosporidium* is not an important zoonotic reservoir for companion animals, as they are most commonly infected with the species *C. canis* and *C. felis* that are adapted to their host animals [41]. With *C. suis*, *C. muris* are responsible for sporadic infections to humans, most likely through direct connection. [42].

12. Significance of *Cryptosporidium*

The early 1980s were the first time that *Cryptosporidium* spp were recognized as a pathogen causing acute gastroenteritis, abdominal pain, and diarrhea [43]. According to Putignani & Menichella, [43], cryptosporidiosis is primarily transmitted through feces, oral excreta, zoonotic, and anthroponotic routes. It has been reported that oocysts are highly robust, highly resistant to environmental changes, and are capable of remaining dormant for up to six months [44]. Infected humans and other animals are capable of shedding millions of oocysts through their bowel movements. In addition, there are no effective therapeutics or vaccines available [45]. The spread of *Cryptosporidium* outbreaks has proven challenging. Farms are a major reservoir of the parasite, which is frequently transmitted to humans via zoonotic transmission [46]. It has been reported that cryptosporidiosis causes damage to the small intestinal microvilli, which predisposes people to infections caused by Rotavirus, and Corona-

viruses, E coli, are rare cases of *Salmonella* [47]. Considering the complex treatment and clinical sign that may result in high death, the prognosis became unfavourable due to the mix enteric infection. When these mixed infections become more severe, lambs die within 2-3 days of the onset of diarrhoea [48]. Approximately two weeks are required for *Cryptosporidium* infection in calves to develop [49]. During *Cryptosporidium* diarrhea, villus atrophy and microvillus shortening or destruction are believed to be caused by parasite invasion and epithelial destruction. This results in impaired nutrient digestion and transportation. The most common clinical manifestations in cattle of *C. Parvum* is diarrheal, depression and abdominal pain [50]. It varies greatly from calves to calves in terms of severity and duration. Mild to severe diarrhea, characterized by pale yellow mucus, can last for up to two weeks. There is usually a feeling of lethargy, anorexia, and dehydration in calves. Dehydration and cardiovascular collapse are often the causes of death for calves in severe cases [51, 52], indicates that human cryptosporidiosis has been associated with farms and exposure to livestock infected by cryptosporidiosis. Indirectly transmission of *Cryptosporidium* in human's cattle cause via water, as it may have been contracted by farm workers or visitors to farms by direct contact [53]. Frequently, livestock are the source of contaminating isolates in the investigation of waterborne outbreaks of cryptosporidiosis. Olson *et al* [52] asserted that run-off from cattle pastures was the predisposing factor, but such conclusions were often only circumstantial.

13. Laboratory diagnosis of cryptosporidiosis

Laboratory diagnosis is necessary for analysis of *Cryptosporidium*, but for identifying outbreaks, tracking sources, and assessing risks, environmental samples are usually necessary for this purpose. It is more common for feces to be examined, however microscopic examination, *Cryptosporidium* can also be detected by MZN dye technique. Antigen and antibody can be detected using immunological methods. Recently, polymerase chain reaction is one of the technique to diagnose the morphology of *Cryptosporidium* [55]. An examination of stool samples is used to diagnose cryptosporidiosis. *Cryptosporidium* may need to be detected in several stool samples over the course of several days. It is most commonly observed that stool specimens are examined microscopically using different techniques for detection of *Cryptosporidium* sp. antigens. It is becoming increasingly common for reference diagnostic laboratories to use molecular methods in order to identify *Cryptosporidium* species at a molecular level. Clinical laboratories rarely perform tests for *Cryptosporidium*; therefore, healthcare professionals should request testing for this parasite specifically. Acid-fast staining techniques are frequently employed in clinical

laboratories, either with or without stool concentrations. It is recommended that immunofluorescence microscopy (followed by enzyme immunoassays) is used for maximum sensitivity and specificity and primarily research tool that uses molecular methods. A stool specimen that contains oocysts (fresh or in storage media) remains infectious for an extended period of time. The stool sample kept in 10% acetate sodium buffer to reduce oocysts and this technique is adopted now to reduce the contamination of *Cryptosporidium* [53].

14. Clinical Feature of *Cryptosporidium*

A relevant epidemiological role could be played by cattle in the transmission of *Cryptosporidium* species infection [53]. There is a relationship between the clinical signs of these animals and parasite responsible for the infection. Anorexia, yellowish diarrhea, and weight loss are the most common symptoms [51]. It has been observed that bovines are affected by Cryptosporidiosis. The infection of the abomasum with *Cryptosporidium andersoni* can cause asymptomatic calves, while the infection with *Cryptosporidium bovis* or cervid genotypes can result in asymptomatic adults. The symptoms of *Cryptosporidium* weight loss, depression and diarrheal and mostly in more than 30 days' age species. The majority of lactating calves are affected, with morbidity and mortality rate.



Figure 1. The figure taken by "Outbreak of Cryptosporidiosis in calves in southern Rio Grande do Sul" by S. F. Vargas Júnior.

In November 2012, A batch of 400 calves was infected with the disease of Rio Grande. Among them, 8.75% developed illnesses and 4% died. Mostly females are occurred under the age of 30-40 days. As shown in Figure 1, represent the different coloured diarrhea after birth. The animals died when clinical signs manifested. It has been reported that around 70 calves died in year 2011 with similar resemble to the clinical symptoms, despite various attempts to diagnose the condition using parasitological tests as well as faecal cultures with no success [56].

Microscopic methods using fecal samples are currently the most widely used for diagnosing this parasitological and pathogenic disease. Farrant *et al.* [57], reported 38 % of calf calves up to 2 months of age demonstrated diarrhea caused through *Cryptosporidium*.

15. Treatment of cryptosporidiosis

In spite of *Cryptosporidium*'s recognition as an important human pathogen, only a limited number of chemotherapeutic agents are available, and there is a need for more research to identify compounds that may be effective against cryptosporidiosis and giardiasis. A number of clinical, trials have shown that nitazoxanide effectively decreases the length of diarrhoea and oocyst, shedding in immune-competent individuals with cryptosporidiosis [57]. Clinically Nitazoxanide are the efficient formula to control the AIDS patient. In this type of patient, it is recommended to administer 2-g per day over a period of 12 weeks [42]. For the treatment of giardiasis, a wide variety of compounds are available. It is commonly used to prescribe nitroimidazol derivatives and a series of side effect occurred when use metronidazole more than 260 mg in a day. Adults treated with tinidazole at a dose of two grams have achieved levels of effectiveness ranging from 80% to 100% and have experienced fewer side effects than those associated with metronidazole.

16. Conclusion

In conclusion, the risk factors associated with bovine cryptosporidiosis in diarrheic calves are multifactorial, involving a combination of environmental, management, and host-related factors. Key risk factors include poor hygiene, overcrowding, insufficient colostrum intake, and younger age of calves, as well as contaminated water and feed sources. Additionally, immunocompromised calves and those exposed to mixed infections are more vulnerable to *Cryptosporidium* infections. Effective control strategies should focus on improving sanitation, ensuring adequate colostrum intake, reducing calf-to-calf transmission through proper housing and spacing, and maintaining good herd health practices. Regular monitoring of water and feed quality, as well as early detection and treatment of diarrheal outbreaks, are essential to reducing the prevalence and severity of cryptosporidiosis in calves. Given the zoonotic potential of *Cryptosporidium*, proactive measures should also consider the health risks to farm workers and other animals, emphasizing the importance of comprehensive biosecurity and hygiene practices on farms. Further research is needed to explore potential vaccination strategies and to better understand the environmental survival and transmission dynamics of *Cryptosporidium*.

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References

- [1]. Fayer, R., Morgan, U. and Upton, S.J., 2000. Epidemiology of *Cryptosporidium*: transmission, detection and identification. *International journal for parasitology*, 30(12-13), pp.1305-1322.
- [2]. Wegayehu, T., Adamu, H. and Petros, B., 2013. Prevalence of Giardia duodenalis and *Cryptosporidium* species infections among children and cattle in North Shewa Zone, Ethiopia. *BMC infectious diseases*, 13(1), pp.1-7.
- [3]. Morris, A.J., Noble, S., Lindo, J.F. And Ong, C.S., 2022. First Report of Cryptosporidium Spp. In Cattle from Jamaica.
- [4]. Khushdil, A., Murtaza, F. And Chattha, M.N., 2016. Cryptosporidiosis Among Children of District Skardu, Pakistan. *Journal of Ayub Medical College Abbottabad*, 28(3), Pp.575-577.
- [5]. Shirley, D.-A. T.; Moonah, S. N.; Kotloff, K. L. Burden of Disease from Cryptosporidiosis. *Curr. Opin. Infect. Dis.* 2012, 25 (5), 555.
- [6]. Seyoum, Z., Getnet, K., Chanie, M., Derso, S. and Fentahun, S., 2018. Morbidity parameters associated with gastrointestinal tract nematodes in sheep in dabat district, northwest Ethiopia. *BioMed Research International*, 2018.
- [7]. Ryan, U.M., Feng, Y., Fayer, R. And Xiao, L., 2021. Taxonomy and Molecular Epidemiology of Cryptosporidium and Giardia—A 50 Year Perspective (1971–2021). *International Journal for Parasitology*, 29(8), Pp.1269-1287.
- [8]. De Graaf, D.C., Vanopdenbosch, E., Ortega-Mora, L.M., Abbassi, H. And Peeters, J.E., 1999. A Review of the Importance of Cryptosporidiosis in Farm Animals. *International Journal for Parasitology*, 29(8), Pp.1269-1287.
- [9]. Caffarena, R.D., Casaux, M.L., Schild, C.O., Fraga, M., Castells, M., Colina, R., Maya, L., Corbellini, L.G., Riet-Correa, F. And Giannitti, F., 2021. Causes Of Neonatal Calf Diarrhea And Mortality In Pasture-Based Dairy Herds In Uruguay: A Farm-Matched Case-Control Study. *Brazilian Journal Of Microbiology*, 52(2), Pp.977-988.
- [10]. Farrant, O., Marlais, T., Houghton, J., Goncalves, A., Teixeira Da Silva Cassama, E., Cabral, M.G., Nakutum, J., Manjuba, C., Rodrigues, A., Mabey, D. And Bailey, R., 2020. Prevalence, Risk Factors and Health Consequences of Soil-Transmitted Helminth Infection On the Bijagos Islands, Guinea Bissau: A Community-Wide Cross-Sectional Study. *PLoS Neglected Tropical Diseases*, 14(12), p. e0008938.
- [11]. Khan, N.U., Usman, T., Sarwar, M.S., Ali, H., Gohar, A., Asif, M., Rabbani, F., Khan, R.U., Sultana, N., Khan, N.A. and Mobashar, M., 2022. The prevalence, risk factors analysis and evaluation of two diagnostic techniques for the detection of Cryptosporidium infection in diarrheic sheep from Pakistan. *Plos one*, 17(7), p.e0269859.
- [12]. Sateesh, A.G., Patil, N.A., Vivek, R.K., Dilipkumar, D. And Basavaraj Awati, R.B., 2021. Study On Diagnosis of Bovine Cryptosporidiosis.
- [13]. Abrahamsen, M. S., T. J. Templeton, S. Enomoto, J. E. Abrahante, G. Zhu, C. A. Lancto, M. Deng, C. Liu, G. Widmer, S. Tzipori, G. A. Buck, P. Xu, A. T. Bankier, P. H. Dear, B. A. Konfortov, H. F. Spriggs, L. Iyer, V. Anantharaman, L. Aravind, and V. Kapur.2004. Complete genome sequence of the apicomplexan, *Cryptosporidium parvum*. *Science*304:441-445.
- [14]. Khushdil, A., Murtaza, F. And Chattha, M.N., 2016. Cryptosporidiosis Among Children of District Skardu, Pakistan. *Journal of Ayub Medical College Abbottabad*, 28(3), Pp.575-577.
- [15]. Shafiq, M.A.B., Maqbool, A., Khan, U.J., Lateef, M. And Ijaz, M., 2015. Prevalence, Water Borne Transmission and Chemotherapy of Cryptosporidiosis in Small Ruminants. *Pakistan Journal of Zoology*, 47(6).
- [16]. Troeger, C., Blacker, B.F., Khalil, I.A., Rao, P.C., Cao, S., Zimsen, S.R., Albertson, S.B., Stanaway, J.D., Deshpande, A., Abebe, Z. and Alvis-Guzman, N., 2018. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Infectious Diseases*, 18(11), pp.1211-1228.
- [17]. Nasir, A., Avais, M., Khan, M.S. And Ahmad, N., 2009. Prevalence of Cryptosporidium Parvum Infection in Lahore (Pakistan) And Its Association with Diarrhea in Dairy Calves. *Int J Agric Biol*, 11(2), Pp.221-224.
- [18]. Khan, S.A., Khan, I., Ali, I., Khan, A.M., Anees, M., Khan, K.A., Rashid, W. And Akbar, N., 2021. Molecular Detection of Cryptosporidium: An Emerging Parasite in Different Water Sources of 2010 Flood-Affected District Nowshera,

Pakistan. *Archives of Microbiology*, 203(7), Pp.4397-4403.

[19]. Fayer, R., 2007. General biology. In *Cryptosporidium and cryptosporidiosis* (pp. 1-42). CRC press.

[20]. Bern, C., Hernandez, B., Lopez, M.B., Arrowood, M.J., De Merida, A.M. And Klein, R.E., 2000. The Contrasting Epidemiology Of Cyclospora And Cryptosporidium Among Outpatients In Guatemala. *The American Journal Of Tropical Medicine And Hygiene*, 63(5), Pp.231-235.

[21]. Lefay, D., Naciri, M., Poirier, P. and Chermette, R., 2000. Prevalence of Cryptosporidium infection in calves in France. *Veterinary Parasitology*, 89(1-2), pp.1-9.

[22]. Gajadhar, A.A., Scandrett, W.B. And Forbes, L.B., 2006. Overview of Food-And Water-Borne Zoonotic Parasites at The Farm Level. *Rev Sci Tech*, 25(2), Pp.595-606.

[23]. Hosseini, S.H., Fathi, S. and Jalousian, F., 2018. Helminths of veterinary and public health importance in Iran: the great neglected parasitic diseases as important challenge for the country. *Iran J Vet Med*, 12, pp.2-7.

[24]. Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D., 2007. Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th. London, UK.

[25]. Preiser, G. *et al.* (2003) An outbreak of cryptosporidiosis among veterinary science students who work with calves. *J. Am. Coll. Health* 51, 213–215

[26]. Huetink, R.E.C., Van der Giessen, J.W.B., Noordhuizen, J.P.T.M. and Ploeger, H.W., 2001. Epidemiology of Cryptosporidium spp. and Giardia duodenalis on a dairy farm. *Veterinary parasitology*, 102(1-2), pp.53-67.

[27]. Rossignol J-F. *Cryptosporidium and Giardia*: treatment options and prospects for new drugs. *Experimental Parasitology*. 2010; 124:45-53.

[28]. Mac Kenzie, W.R., Schell, W.L., Blair, K.A., Addiss, D.G., Peterson, D.E., Hoxie, N.J., Kazmierczak, J.J. And Davis, J.P., 1995. Massive Outbreak of Waterborne Cryptosporidium Infection in Milwaukee, Wisconsin: Recurrence of Illness and Risk of Secondary Transmission. *Clinical Infectious Diseases*, 21(1), Pp.57-62.

[29]. Adam, R.D., 2001. Biology of Giardia Lamblia. *Clinical Microbiology Reviews*, 14(3), Pp.447-475.

[30]. Chalmers, R.M., Ferguson, C., Cacciò, S., Gasser, R.B., El-Osta, Y.G.A., Heijnen, L., Xiao, L., Elwin, K., Hadfield, S., Sinclair, M. and Stevens, M., 2005. Direct comparison of selected methods for genetic categorisation of *Cryptosporidium* parvum and *Cryptosporidium hominis* species. *International journal for parasitology*, 35(4), pp.397-410.

[31]. Tuzio, H., Edwards, D., Elston, T., Jarboe, L., Kudrak, S., Richards, J., Rodan, I. And American Association of Feline Practitioners, 2005. Feline Zoonoses Guidelines from The American Association of Feline Practitioners. *Journal of Feline Medicine and Surgery*, 7(4), Pp.243-274.

[32]. Carmena, D., 2010. Waterborne transmission of *Cryptosporidium* and Giardia: detection, surveillance and implications for public health. *Current research, technology and education topics in applied microbiology and microbial biotechnology*, 20, pp.3-4.

[33]. Perch, M., Sodemann, M., Jakobsen, M.S., Valentiner-Branth, P., Steinsland, H., Fischer, T.K., Lopes, D.D., Aaby, P. and Mølbak, K., 2001. Seven years' experience with cryptosporidium parvum in Guinea-Bissau, West Africa. *Annals of tropical paediatrics*, 21(4), pp.313-318.

[34]. Shirley, D.-A. T.; Moonah, S. N.; Kotloff, K. L. Burden of Disease from Cryptosporidiosis. *Curr. Opin. Infect. Dis.* 2012, 25 (5), 555.

[35]. Thomson, S.; Hamilton, C. A.; Hope, J. C.; Katzer, F.; Mabbott, N. A.; Morrison, L. J.; Innes, E. A. Bovine Cryptosporidiosis: Impact, Host-Parasite Interaction and Control Strategies. *Vet. Res.* 2017, 48 (1).

[36]. Rebhun W.1995. Diseases of the uuminates. In Diseases of dairy cattle, Williams & Wilkins co., Baltimore, Philadelphia, Hong Kong, London, Munich, Sydney, Tokyo, pp. 168-169.

[37]. Coop RL, Wright SE.2000. Cryptosporidiosis. In Diseases of Sheep, 3rd edn, Eds Martin, W. and Aitken, I., pp. 153-155, Blackwell Science, Oxford.

[38]. Ralston, B.J. *et al.* (2003) Prevalence and infection pattern of naturally acquired giardiasis and cryptosporidiosis in range beef calves and them

[39]. De Graaf, D.C., Vanopdenbosch, E., Ortega-Mora, L.M., Abbassi, H. And Peeters, J.E., 1999. A Review of the Importance of Cryptosporidiosis in Farm Animals. *International Journal for Parasitology*, 29(8), Pp.1269-1287.

[40]. Olson, M.E., O'Handley, R.M., Ralston, B.J., McAllister, T.A. and Thompson, R.A., 2004. Update on *Cryptosporidium* and Giardia infections in cattle. *Trends in parasitology*, 20(4), pp.185-191.

[41]. Monis, P.T. And Thompson, R.C.A., 2003. Cryptosporidium and Giardia-Zoonoses: Fact or Fiction? *Infection, Genetics and Evolution*, 3(4), Pp.233-244.

[42]. Maurya, P.S., Rakesh, R.L., Pradeep, B., Kumar, S., Kundu, K., Garg, R., Ram, H., Kumar, A. and Banerjee, P.S., 2013. Prevalence and risk factors associated with *Cryptosporidium* spp. infection in young domestic livestock in India. *Tropical Animal Health and Production*, 45(4), pp.941-946.

[43]. Vohra, P., Sharma, M. and Chaudhary, U., 2012. A comprehensive review of diagnostic techniques for detection of *Cryptosporidium parvum* in stool samples. *J Pharm*, 2(5), pp.15-26.

[44]. Ryan, Una M., Yaoyu Feng, Ronald Fayer, and Lihua Xiao. "Taxonomy and molecular epidemiology of *Cryptosporidium* and *Giardia*—a 50-year perspective (1971–2021)." *International Journal for Parasitology* (2021).