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Review Article

Review on Bovine Cryptosporidiosis, its Associated Risk Factors and Diagnostics Methods

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Abstract

Bovine cryptosporidiosis, caused by the protozoan parasite Cryptosporidium, poses a significant threat to cattle health and productivity worldwide. This review provides a comprehensive analysis of bovine cryptosporidiosis, focusing on its associated risk factors and diagnostic methods. Understanding the risk factors contributing to the prevalence and transmission of Cryptosporidium in cattle populations is crucial for effective disease management. Factors such as age, herd size, housing conditions, environmental contamination, and geographic location play pivotal roles in the epidemiology of bovine cryptosporidiosis. Additionally, interactions between host susceptibility and pathogen virulence further complicate disease dynamics.

Accurate and timely diagnosis is essential for implementing control strategies and mitigating the economic losses associated with bovine cryptosporidiosis. Various diagnostic methods, including microscopy, immunological assays, molecular techniques such as PCR (Polymerase Chain Reaction), and emerging technologies like loop-mediated isothermal amplification (LAMP), are discussed in detail. Each method's sensitivity, specificity, advantages, and limitations are evaluated, providing insights into their practical utility in different settings. Furthermore, advancements in diagnostic tools and techniques, such as high-throughput sequencing and proteomic approaches, offer promising avenues for enhancing our understanding of Cryptosporidium diversity, pathogenesis, and drug resistance patterns. Integrating these innovative approaches with traditional diagnostic methods holds great potential for improving disease surveillance and control strategies.

The aim of this review is to highlights the multifaceted nature of bovine cryptosporidiosis and underscores the importance of a holistic approach encompassing risk factor identification, accurate diagnostics, and targeted interventions for effective disease management in cattle populations. Continued research efforts aimed at elucidating the complex interactions between host, parasite, and environment will be instrumental in developing sustainable control measures to mitigate the impact of bovine cryptosporidiosis on animal health and welfare.

Keywords: Calves, Cryptosporidium, Parvum, Prevalence

Abbreviations: BKIs: Bumped Kinase Inhibitors; CDC: Center for Disease Control and Prevention; DFA: Direct Fluorescence Antibody; DMSO: Dimethyl Sulfoxide; DNA: Deoxyribonucleic Acid; EIAs: Enzyme Immunoassays; ELISA: Enzyme-Linked Immunosorbent Assay; IgA: Immunoglobulin A; IgG: Immunoglobulin G; MZN: Modified Ziehl Neelsen; PCR: Polymerase Chain Reaction; PPV: Positive Predictive Value; RT: Reverse Transcription; SSU: Small Sub Unit

Introduction

Cryptosporidiosis is an infectious disease of relevance to the cattle industry because it has the potential to result in economic and production loss directly through cattle mortality and indirectly through decreased milk production, increased susceptibility to other diseases, treatment costs, and poor growth [1,2]. In addition, there is a known risk of zoonotic transmission of Cryptosporidium in cattle to humans, with a resulting impact on public health [3].

Cryptosporidium is a ubiquitous protozoan parasite and the causal agent of cryptosporidiosis, a potentially lethal diarrheal disease that is known to affect cattle and a wide range of hosts [4]. Of the 26 species of Cryptosporidium currently identified, cattle are the primary hosts of Cryptosporidium andersoni, Cryptosporidium bovis, Cryptosporidium ryanae, and Cryptosporidium parvum. Epidemiologically, C. parvum is often regarded as the most important species because of its primary role in the occurrence of cryptosporidiosis in young calves [5]. Cattle infections are spread by the Faecal-oral route, where oocysts are transmitted from host to host directly or indirectly through contaminated environment such as water and food supplies [1].

Neonatal diarrhea is mostly caused by Cryptosporidium, which primarily affects pre- to post-weaned dairy calves [6]. Adult cattle can get infected, but these infections are frequently minor or asymptomatic [4,6]. According to Abeywardena et al. [4], clinical symptoms in cattle can range from acute to chronic diarrhoea and include dehydration, fever, anorexia, weight loss, depression, dullness, and bloating. Complications occasionally lead to fatalities [7], and it's also a major cause of diarrhoea and gastrointestinal illness had been reported [8,9]. Studies conducted in parts of the world also stated higher prevalence rates ranging from 27% to 86.7% had been reported [13]. Numerous factors render cryptosporidiosis one of the most famous parasitic infections worldwide. Among these factors, the parasites have high infectivity to animal species associated with high mortality in the immunocompromised host [10,11].

The associated factors of Cryptosporidium infection were generally factors related to farm location and management and factors related to water supply and sanitation [12]. Among factors related to farm location and management: intensive farming, urban location of farms, medium

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herd size, absence of calving pen, absence of calf bedding, dam suckling and weaning age ≥ 6months. Whereas, among factors related to water supply and sanitation, river/stream water sources, limited access to drinking water, disposal of farm waste water to wells, occurrence of other diseases (Foot and Mouth Disease and Pasteurellosis), group penning, unclean pens and unclean tail, hindquarter and flank of animals [13].

The detection of Cryptosporidium species can be accomplished using a variety of techniques, including microscopic, immunological, and molecular techniques. Microscopic detection is based on identifying the oocysts in fecal samples that are resistant to chemicals and the environment [14]. Ziehl-Nielsen stained fecal smears, in which the sporozoites appear as bright red granules, can be used to demonstrate oocysts [15]. The proportional occurrence of viral enteropathogens and C. parvum in samples from diarrheal calves in dairy calves emphasized the growing significance of Cryptosporidium, which requires further examination [16].

In either human or veterinary medicine, there is no treatment for it. But according to Van Voorhis et al. [17], nitazoxanide and halofuginone are approved drugs for the treatment of prophylaxis and metaphylaxis, respectively. Controlling cryptosporidiosis has required cutting off the transmission routes of Cryptosporidium species that primarily infect humans or those that infect animals and spread to humans (zoonotic). (Anthroponotic). However, there is little to no documented information available in many countries around the world to begin investigations and prepare for control actions. Similar to Ethiopia, there was a scarcity of well-documented reviews on the prevalence, its associated risk factors, and diagnostic procedures for bovine cryptosporidiosis despite reports that the infection was highly widespread there. This review paper's goal is to examine bovine cryptosporidiosis, its contributing causes, and diagnostic techniques.

Literature Review

Historical Background of Cryptosporidium

The first case of cattle being infected with a Cryptosporidium species was documented in the early 1970s [18]. However, until 1980 [19], the significance of Cryptosporidium species as main enteropathogens was questionable due to their interaction with other viral or bacterial enteropathogens. The ability to isolate infective oocysts from other contaminating pathogens was made possible in the years that followed, allowing for the experimental demonstration that Cryptosporidium may cause clinical diarrhoea in calves [20].

Etiology and Taxonomy of Cryptosporidium

Cryptosporidium species are classified under the family Cryptosporidiidae, sub-order Eimeriorina, order Eucoccidiorida, sub-class Coccidiasina, class Sporozoasida, phylum Apicomplexa [21]. Among described species of Cryptosporidium, the most species infecting animals and man include Cryptosporidium andersoni, C. bovis, C. canis, C. felis, C. hominis, C. meleagridis, C. molnari, C. parvum, C. scophthalmi, C. scrofarum, C. suis and C. xiaoi. Also, four species of Cryptosporidium are commonly found in calves; C. parvum, C. bovis, C. andersoni and C. ryanae but only C. parvum is associated with clinical disease in neonatal calves, with older animals showing asymptomatic shedding of Oocysts [22].

The naming of Cryptosporidium species is undergoing rapid changes. The early classification of Cryptosporidium relying on host occurrence, and lacking morphological characters to differentiate variants, created a huge debate on species organization. Moreover, it was not obvious to understand whether phenotypic differences were a consequence of genetic differences or a result of host or environmental induced changes. However, molecular characterization of Cryptosporidium helped to clarify the confusion in Cryptosporidium taxonomy [23].

For a very long period, a clear species classification was impossible due to the exogenous oocyst stage's absence of distinguishing morphological traits. For the purpose of identifying and naming the species of Cryptosporidium, researchers used the morphology of the endogenous and exogenous developmental phases, preference sites, and host specificity [24]. Originally only one species, C. parvum was identified and split into two host-adapted genotypes: type 1 genotype, or "human genotype" (H type), and type 2 genotype, or "cattle genotype" (C type). Eventually, these genotypes were separated into two distinct species, C. hominis (formerly type 1) and C. parvum (formerly type 2), based on their host-adaptation levels [23].

Cryptosporidium taxonomy had been under a continual review process and many of the host-adapted genotypes were given species status based on the standardized guideline that includes:- morphometric data on oocysts, genetic characterization, natural, and when feasible, experimental host specificity, and compliance with International Commission on Zoological Nomenclature rules [25].

Life Cycle of Cryptosporidium

Cryptosporidia are protozoa with a complex monoxenous development life cycle which can be divided into an asexual (sporogony and schizogony/merogony) and a sexual (gamogony) phase. The infective stage of the parasite, the oocyst, contains four small banana-shaped sporozoites. After ingestion of the oocyst, excystation processes is enhanced at 37°C and likely involve parasite derived enzymes [26]. Zoite motility requires discharge of adhesive molecules from the apical complex and is temperature, Ca++ ion, and parasite cytoskeleton dependent. Ultimately, the apical end of the parasite attaches to the epithelial cell and initiates the internalization process. The result of excystation, attachment, and internalization is a fully encapsulated parasite or trophozoite that undergoes cell divisions resulting in six to eight new banana-shaped parasites (merozoites) within a Type I meront [26].

These merozoites can re-infect the epithelium and form either a type I meront, effectively escalating the infection, or a type II meront, destined for sexual reproduction. The merozoites derived from type II meronts reinfect the epithelium and differentiate into either a micro- or macrogamonts. The microgamonts fertilize macrogamonts resulting in the zygote, the only diploid developmental stage of the parasite. The diploid zygote undergoes a process similar to meiosis and forms either a thin or thick walled oocyst [27]. Most of the mature zygotes (around 80%), develop a tough outer cover measuring 2.5–5 μm in diameter, become infective oocysts and exits the host through faeces to contaminate the environment. The rest of the mature zygotes having only a thin outer membrane, excysts in the gut lumen and re-infects the host [26]. During the invasion of the free living stages, proliferation and differentiation take place within a unique parasitophorous vacuole under the host cell brush border, but outside the host cell cytoplasm [28]. At the parasite-cell interface, Cryptosporidium forms an actin-rich disk, a feeder organelle that is thought to be a small channel funneling into the host cell cytoplasm and responsible for intake of nutrition [27].

Epidemiology of Cryptosporidium

Geographic distribution

According to reports, Cryptosporidium parvum can infect both humans and ruminant cattle, particularly newborn calves under 2 months old [29]. In comparison to dairy calves, the prevalence is frequently lower in beef calves [30]. Calves in India, Pakistan, and Chile have prevalence rates of 16.3% [31], 27.2% [29], and 57.9% [32], respectively. Cattle in Myanmar were found to have an overall prevalence of 14%, with calves younger than 6 months possessing a much higher proportion rate [33]. The parasite's oocysts were found in around one-third of the dairy cattle farms in central Thailand [34].

Cryptosporidium parvum infections of calves are considered

endemic globally. Prevalence and severity of disease peak in the second week of life. Endogenous stages infect enterocytes of the distal small intestine, caecum and colon. Affected animals usually recover within 2 weeks of showing signs of illness. Clinical signs can range from a mild to in apparent infection in older animals to severe scouring in young animals, and can cause varying degrees of dehydration, dullness, anorexia, fever and loss of condition. Mortality is generally low unless occurring as a mixed infection with other enteric pathogens such as Escherichia coli or rotavirus, although severe outbreaks of cryptosporidiosis are sometimes reported [35].

Cryptosporidia species affecting cattle differ from country to country, Cryptosporidium andersoni infect mainly mature cattle, and C. bovis and the Cryptosporidium deer-like genotype infect older dairy calves [5,36]. C. bovis and C. ryanae were encountered in cattle in Malaysia [37], while a study in Turkey reported that C. parvum was the only species encountered in cattle [38]. A wide range of calf (0-59%) and farm (0-100%) prevalence estimates have been reported from different countries [39].

The following characteristics have a significant impact on the epidemiology of infections: low infectious dose; immediate infectiousness of oocysts upon excretion in feces; multiple routes of transmission; high stability and ability to survive for weeks to months in the environment; potential for contamination of water and food due to environmental dispersal. Numerous conditions, such as large population densities, intimate contact with diseased hosts, polluted water, or food, enhance the direct or indirect spread of these illnesses. These variables rely on the animal that is transmitting the disease, whether it be through zoonotic or anthroponotic transmissions [40].

Epidemiology of Cryptosporidium infection in calves in Ethiopia

Research on cryptosporidiosis in dairy calves in Ethiopia remains limited, though existing studies have shed light on its prevalence across different regions of the country. Several investigations have been conducted, primarily in the central region, indicating the occurrence of Cryptosporidium infection in dairy farms. Prevalence rates within this region have been documented to range from 7.2% to 15.8% in various studies [12,41,42]. A broader study encompassing nine regions of Ethiopia reported an overall prevalence of 2.3% [41]. Additionally, a longitudinal study conducted in both small and large scale dairy farms situated in Debre-Zeit by Wudu (2004) aimed at understanding the causes of calf morbidity and mortality, revealed a prevalence of Cryptosporidium at 6.7% among diarrheic calves aged 20 to 90 days. These findings collectively underscore the presence of Cryptosporidium infection in Ethiopian dairy farms and emphasize the need for further research and targeted interventions to address this issue effectively (Table 1).

Table 1: Studies on prevalence of *Cryptosporidium* infection in cattle in Ethiopia

Study site	No of samples	Prevalence (%)	References
Central Ethiopia	580 calves	17.6.	Abebe et al. [12]
Nine regions	350 cattle	2.3	Adamu et al. [76]
Eastern Ethiopia	133 calves	27.8	Alemayehu et al. [77]
North Shewa Zone	384 cattle	7.8	Wegayehu et al. [42]
Bishoftu, Oromia	214 calves	13.6	Dinka and Berhanu (2015)
Central Ethiopia	378 cattle	10.9	Gashaw et al. [78]
Southern Ethiopia	330 Calves	13.0	Hailu et al. [79]
Central Ethiopia	270 Calf	14.8	Manyazewal et al. [13]

Major Risk Factors for Cryptosporidium infection in calves

Major risk factors for Cryptosporidium infection in calves include various aspects of farm management, environmental conditions, and animal health. These factors contribute to the prevalence and transmission of the parasite within cattle populations. Here are some of the significant risk factors.

Age of Calves

Young calves, especially those less than three weeks old, are particularly susceptible to Cryptosporidium infection due to their immature immune systems and higher likelihood of exposure to contaminated environments [43]. Although exceptions occur, older animals generally develop poor infections, even when unexposed previously to this parasite. Several investigations revealed age as an important risk factor associated with Cryptosporidium infection in cattle [13,44-46]. In a study by Ayele et al. [47], Cryptosporidium infection occurrence was 2.12 times more in the young calf (6 months) Zeleke et al. [48], also indicated the prevalence of Cryptosporidium infection decreases as the age of animal's increases.

Housing Conditions

Overcrowded or poorly ventilated housing facilities increase the risk of Cryptosporidium transmission among calves. Close contact between infected and susceptible animals facilitates the spread of the parasite [49].

Hygiene Practices

Inadequate hygiene practices, such as failure to regularly clean and disinfect calf pens and feeding equipment, contribute to the persistence of Cryptosporidium oocysts in the environment and increase the likelihood of calf exposure [50].

Water Quality

Contaminated water sources, including drinking water and stagnant puddles, serve as reservoirs for Cryptosporidium oocysts. Calves that consume contaminated water are at a higher risk of infection [51].

Feeding Practices

Feeding colostrum and milk from infected cows or using contaminated milk replacers can introduce Cryptosporidium oocysts into the calf's gastrointestinal tract [52].

Climate and Seasonality

Environmental factors such as temperature and humidity influence the survival and transmission of Cryptosporidium oocysts. Warmer and wetter conditions may favor the persistence and spread of the parasite [53].

Herd Size

Larger cattle herds may experience higher rates of Cryptosporidium infection due to increased opportunities for contact between infected and susceptible animals [54].

Presence of Other Diseases

Concurrent infections or underlying health conditions, such as diarrhea caused by other pathogens, can weaken calves' immune systems and make them more susceptible to Cryptosporidium infection [55].

Management and hygienic conditions:

The risk of Cryptosporidium infection increases when animals are communally housed and overcrowded [56], and when hygiene and certain other management practices are deficient [57].

Resistance of the oocysts

Cryptosporidium oocysts are extremely resistant to environmental factors and to the action of chemical agents commonly used including chlorine and therefore, they are able to survive in the environment-bedding, walls, feeding troughs, drinking units, utensils, and maintain their infective capacity for prolonged periods of time [58].

Pathogenesis and Clinical Symptoms in Calves

The intestinal Cryptosporidium species, C. bovis and C. parvum, complete their life cycle in the ileum, and occasionally in the colon, caecum, and duodenum. The pathogenicity of C. bovis is believed to be limited, probably due to immunity in older calves. In young calves, C. parvum is considered a highly pathogenic species. The invasion and colonisation of the epithelial surface by the different parasitic stages results in the loss of epithelial cells and the microvillus brush border. Furthermore, the epithelial tight junctions are disrupted leading to increased epithelial permeability, decreased intestinal surface area, impaired nutrient and electrolyte transport as well as loss of membrane-bound digestive enzymes such as lactase [59].

Cryptosporidium associated diarrhoea is caused by two pathogenic mechanisms. Malabsorptive diarrhoea is caused by loss of enterocytes and blunting of villi, which reduces the intestinal surface and presence of mature cells, leading to decreased nutrient and water absorption [60,61]. Prostaglandins induce secretion of chloride and carbonate ions into the intestinal lumen, and decrease absorption of sodium chloride; this produces an osmotic pressure that forces water into the lumen, resulting in secretory diarrhoea [60]. Intestinal damage caused by massive infection may lead to reduced growth rates; however, intestinal absorption was restored three weeks post-infection, indicating that no prolonged or permanent damage occurs [61].

In histopathological preparations villus shortening and fusion, as well as crypt hyperplasia and an increase in intra-epithelial lymphocytes can be observed [62]. Clinical symptoms are most frequently observed in calves between the age of 5 days and 1 month, it includes Malabsorptive and secretory diarrhoea which is usually self-limiting within 2 weeks. The diarrhoea can be mild to severe with pale to yellowish watery or mucoid faeces. Calves can be dehydrated, depressed, and anorectic. The severity and duration of the clinical symptoms are highly variable, depending on concurrent viral, bacterial, or parasitic infections, but also host factors [61].

Cryptosporidium andersoni is a cattle specific species with a prepatent period of 18-45 days [30]. This species invades the peptic and pyloric glands of the abomasum in weaned calves and older cattle causing glandular dilatation and hypertrophy of the gastric mucosa and thinning of the epithelial lining. Infection with C. andersoni does not usually result in pronounced diarrhoea, but mainly causes inhibition of protein digestion due to increased gastric pH and decreased gastric proteolytic activity. This results in maldigestion; moderate to severe impairment of weight gain, decreased feed efficiency, and reduced milk production [63].

Mortality is variable and is most often observed in calves most of the infected calves recover spontaneously but a few may die. On necropsy, the small or large intestine or both may be distended with gas and contain watery yellow fluid. Enteritis and colitis may be apparent. Calves with severe cryptosporidiosis can take several weeks to fully recover, and there is certainly an initial negative impact on production due to weight loss or impaired weight gain, and due to treatment expenses. Whether cryptosporidial infections early in life have long-term detrimental effects is uncertain. Calves that recover from Cryptosporidium-associated diarrhoea usually do not have recurrent clinical infections [63].

Laboratory diagnostic methods

A variety of confirmatory diagnostic techniques has been developed to identify Cryptosporidium oocysts or DNA in stool, intestinal fluid, tissue samples, biopsy specimens, or other biological samples, the methods having high positive predictive value (PPV) include direct fluorescent antibody (DFA), serological tests, polymerase chain reaction (PCR), enzyme immunoassay (EIA), or microscopy with tinctorial and fluorescent stains [64]. Detection of Cryptosporidium antigen by screening test methods such as immunochromatographic card/rapid card test gives a presumptive diagnosis of the infection. In-vitro propagation of the organisms is not possible. Immunofluorescence microscopy is used as the gold standard method of detecting Cryptosporidium oocysts at reference laboratories in the USA and Europe [10], whereas, the modified Ziehl-Neelsen staining is considered the gold standard method by others [14].

Histopathology

Histopathological diagnosis of Cryptosporidium depends on the identification of the 4-6 µm intracellular spherical oocysts (oocyst components) within biopsy specimens of the gastrointestinal mucosa. In haematoxylin and eosin-stained sections, developmental stages of the parasite appear as small, spherical, basophilic bodies (2-5 µm) within the microvillous region of the intestinal mucosa. Transmission electron microscopy can be used to confirm the diagnosis and reveal distinct life cycle forms, each within a parasitophorous vacuole confined to the microvillous region of the host cell [65].

Concentration methods

Stool concentration techniques that are useful for the identification of C. parvum oocysts include flotation of oocysts in Sheather's sugar solution, zinc sulphate (specific gravity 1.18 or 1.2), or saturated sodium chloride (specific gravity 1.27). Stool concentration techniques using sedimentation include formalin-ether and formalin-ethyl acetate. If one is using concentration methods to look for C. parvum oocysts in stool or other body fluid samples, it is advisable to centrifuge at greater than 500 x g for at least 10 min [10].

Staining of Faecal Specimens

Diagnostic sensitivity of microscopic staining methods is often limited by the shedding of organisms intermittently or in low numbers [66]. This sensitivity is also dependent on the skills of the microscope technicians. The differential staining methods for Cryptosporidium include the modified acid-fast Ziehl-Neelsen stain, the negative staining technique of Heine, Safranin stain, trichrome stain, and DMSO-carbol fuchsin [67], which stain the parasite in red/ unstained/ bright reddish-orange and counterstain the background. Auramine rhodamine staining of stool sediment smears followed by modified Ziehl-Neelsen (acid-fast) confirmatory staining is a sensitive and specific approach for the identification of Cryptosporidium oocysts in the stool. Since oocysts of Cryptosporidium species are indistinguishable from one another, molecular methods are essential for the identification of the 37 species, genotypes, and subtypes of Cryptosporidium to specifically identify the organism responsible for the infection and the source and routes of transmission [68].

The modified acid-fast Ziehl-Neelsen stain

This Gold Standard stain for the detection of Cryptosporidium species is classically performed by staining a methanol-fixed thin smear of faecal material with an undiluted carbol-fuchsine solution for at least 15 minutes. Subsequently, the slide is rinsed in tap water and placed in an acid-alcohol solution to remove the stain, while acid-fast structures will resist the acid alcohol destaining action. After rinsing again, the slide is placed for a short period in a counter-staining product, such as methylene blue, providing contrast between background material and acid-fast structures. The slide is rinsed once more and after the slide has been air-dried, it can be examined using x40 eyepieces and an oil-

immersion objective of x100 magnification. Cryptosporidium oocysts will appear as pink-stained, round to oval structures of about 4 to 6 µm in diameter, containing distinct internal structures [69]. The modified acid-fast staining is a time-consuming procedure (about 30 to 45 minutes) and good staining and visual skills are necessary. The modified Ziehl-Neelsen staining is a low-cost technique and provides a permanent stain that makes it possible to send doubtful or scanty positive slides to a reference laboratory for confirmation [8].

The negative staining technique of Heine

In this method, a small amount of faecal matter is mixed with an equal amount of undiluted carbolfuchsine solution on a microscope slide. A thin smear is prepared, allowed to air dry, and examined using x40 eyepieces and an oil-immersion objective of x100 magnification. Cryptosporidium oocysts appear as unstained, strongly refractive, and round to oval structures of about 4 to 6 µm in diameter. Internal structures are slightly visible as darker specks inside the oocyst. The slides should be examined within 15 minutes after they have been air-dried. This time-lapse can be prolonged to 30 minutes by using samples that have been fixed in 10% formalin, before staining. If the slide is not examined within 15 - 30 minutes the oocysts will dry out and become less visible [14].

Safranin stain

Oocysts of Cryptosporidium often (but not always) stain a bright reddish-orange colour. This method, advocated for Cyclospora, is not widely used for Cryptosporidium because Cryptosporidium oocysts may not always properly stain (Control and Prevention, 2015).

Trichrome stain

Oocysts may be detected, but should not be confirmed, by this method. This staining method is inadequate for definitive diagnosis because all oocysts will appear unstained. Oocysts appear as small round structures measuring 4 to 6 µm (Control and Prevention, 2015).

Serological methods

Serological assays are important tools for epidemiological surveillance of Cryptosporidium, as specific antibody responses develop after symptomatic and asymptomatic infections, and can discriminate past, recent, and repetitive infections. Since Immunoglobulin A (IgA) responses are generally short-lived and IgG responses can persist for several months, Antibody to Cp23 appears to correlate with distant infection, responses to Cp17 (gp15) suggest recent infection, and responses to P2 are associated with repeated infection [70].

Molecular detection methods

Molecular methods for detecting Cryptosporidium in clinical specimens are more sensitive than conventional microscopy [71,72]. Different types of molecular techniques have been used to differentiate Cryptosporidium species/genotypes, with the SSU rRNA-based tools being the most employed, especially PCR-RFLP [73].

Polymerase Chain Reaction (PCR)

DNA extracted from oocysts can be amplified by one of several PCR protocols: standard PCR, nested PCR, Reverse Transcription PCR (RT-PCR), real-time PCR, Random Amplified Polymorphic DNA PCR (RAPD-PCR), and Arbitrary Primed PCR (AP-PCR). The nested PCR, PCR-restriction fragment length polymorphism (RFLP), and real-time PCR are the most commonly used methods for the detection of Cryptosporidium and species identification. In standard PCR, one pair of primers is used to amplify a gene in the forward (5'-) and reverse (3'-) directions, whereas in nested PCR two sets of primers are used, of which the first 43 (external) primer pair targets the gene of interest and the second (internal) primer pair amplifies a shorter (internal) segment of the amplicons produced by the primary PCR [73].

Treatment

In calves, prevention and treatment of cryptosporidiosis can be made by Halofuginone lactate drug but cannot be used in animals have shown signs of diarrhea for > 24 hours (does not completely prevent or cure disease) but can reduce Oocysts shedding and the duration of diarrhea, as a prophylactic measure the drug should be given within 48 hours of birth and as a therapeutic agent, within 24 hrs. of the onset of symptoms [22].

According Viu et al. [74], stated several infected calves were treated with 100 mg/kg paromomycin twice daily for 11 days significant reductions in the severity of diarrhea Oocysts shed less than untreated calves were observed. Furthermore, a few coccidiostats, such as decoquinate have been tested against Cryptosporidium in neonatal calves with limited or no reduction in Oocysts. However, more recent studies that have evaluated novel BKIs as a potential treatment for bovine cryptosporidiosis showed that experimentally infected calves treated with BKIs had a reduction in Oocysts shedding when compared with untreated controls [27].

Prevention and control

The Oocysts of Cryptosporidium are very difficult to eliminate from the environment. The preventive measures that can reduce transmission of bovines cryptosporidiosis are effective farm management practices or frustration to reduce fundamental risk factors by preventing the environmental contamination through, regular removal of feces and contaminated bedding from calving areas and calf houses, combined with steam-cleaning and disinfection with a suitable disinfectant such as Hydrogen Peroxide based disinfectants can help to reduce the environmental buildup of Cryptosporidium Oocysts. Also, limiting the amount of animals density in the farms or stocks, minimizing contact between personnel, calves, and other herds, keeping young animals or susceptible hosts that have a high risk of infection separated from adult animals and keeping a short calving period of animals which may decrease the opportunities for C. species to spread within animal herds [22]. At present, there are no commercially available vaccines to prevent cryptosporidiosis in either farm livestock or humans. However, several attempts to develop such a vaccine have been made, some of which were partially successful under experimental conditions. Calves that were immunized with killed (γ-irradiated or lyophilized) showed a reduction in Oocysts shedding and diarrhea than non-immunized calves [75].

The disease frequently arises in the first week of life, trying to immunize the neonatal calves is doubtful to be successful as this will not give enough time to persuade important immune reply before infection hence, immunizing pregnant cows can produce antibodies against infection which can be passed via colostrum to their calves and thus, calves receiving colostrums from cows vaccinated in this method with recombinant C. parvum were reported to protect against diarrhea and also had reduced Oocysts shedding, then those calves received colostrum from non-vaccinated cows [75].

Public Health Importance of Cryptosporidium

An increasing problem that contributes to broad disease outbreaks is waterborne pollution. Today, cryptosporidium is also regarded as a significant food-borne pathogen causing an illness with global socioeconomic relevance. Cryptosporidiosis has become more prevalent in animals as a result of increased environmental pollution and changes in livestock production. Protozoan pathogens are a significant source of gastrointestinal sickness and are having an increasing influence in emerging nations. Up to 20% of all instances of pediatric diarrhea in underdeveloped nations are caused by cryptosporidium, an AIDS complication that can be lethal.

Conclusion and Recommendations

Cryptosporidiosis produces a large amount of oocysts in both infected animals and afflicted humans. In conclusion, the review provides comprehensive insights into bovine cryptosporidiosis, encompassing its associated risk factors and diagnostic methods. Cryptosporidiosis poses a significant threat to cattle health and productivity globally, with various factors influencing its prevalence and transmission dynamics. Factors such as age, herd size, housing conditions, environmental contamination, and geographical location play crucial roles in the epidemiology of the disease. Furthermore, the interaction between host susceptibility and pathogen virulence adds complexity to disease dynamics. Effective disease management strategies rely on accurate and timely diagnosis. This review highlights various diagnostic methods available for detecting Cryptosporidium in cattle, including microscopy, immunological assays, and molecular techniques such as PCR and LAMP. Each method has its advantages and limitations, necessitating careful consideration of factors such as sensitivity, specificity, and practical utility in different settings.

Based on the above findings presented in this review, the following recommendations are proposed:

Recommendations:

- Enhanced Surveillance: There is a need for improved surveillance systems to monitor the prevalence and distribution of bovine cryptosporidiosis. Longitudinal studies covering diverse geographical regions and cattle populations would provide valuable insights into disease trends and emerging risk factors.
- Education and Awareness: Increasing awareness among cattle farmers, veterinarians, and stakeholders about the importance of preventive measures and early detection of cryptosporidiosis is essential. Training programs and educational materials should be developed to promote good husbandry practices and proper hygiene management on dairy farms.
- Integrated Approach: Disease control efforts should adopt an integrated approach that combines preventive measures, such as improved sanitation, biosecurity protocols, and vaccination strategies, with accurate diagnostic tools. Collaboration between researchers, policymakers, and industry stakeholders is critical for implementing effective control measures.
- Research Investment: Continued investment in research and development of new diagnostic technologies and therapeutic interventions is vital for advancing our understanding of bovine cryptosporidiosis. Further investigation into host-pathogen interactions, genetic diversity, and drug resistance patterns will facilitate the development of targeted control strategies.

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