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A REVIEW OF *CRYPTOSPORIDIUM* SPP. INFECTION IN LIVESTOCK

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Abstract

Cryptosporidium infection is a major problem in neonatal ruminants. *Cryptosporidium* is a zoonotic small protozoan parasite (4-6µm) that inhabit microvillus border of epithelium mucosal in many vertebrates including livestock. *Cryptosporidium spp.* is known as one of the most common water and foodborne diseases. It is a common cause diarrhea in human and animals. This review highlights the occurrence of *Cryptosporidium* infection livestock particularly in cattle, goats, sheep and buffaloes are explained in this review paper including the clinical and pathological features and the interaction of *Cryptosporidium* spp. with other enteropathogens. The information about the transmission, epidemiological factors of the infection and economic losses are also discussed in this paper. *Cryptosporidium* infection is a major problem in livestock as the infection causes neonatal diarrhea syndrome. *Cryptosporidium* infections in small ruminants may be a source for cryptosporidiosis in human. Since there is a lack of effective drug available to treat cryptosporidiosis, application of proper effective management is a must to prevent the spread of cryptosporidiosis among the livestock.

Keywords: Neonatal, *Cryptosporidium*, zoonotic, diarrhea, livestock

Abstrak

Jangkitan *Cryptosporidium* adalah masalah utama dalam kalangan neonatal ruminan. *Cryptosporidium* adalah zoonosis koksidia parasit (4-6µm) yang mendiami sempadan mikrovilus epitelium mukosa di dalam kebanyakan vertebrat termasuk haiwan ternakan. Cryptosporidium spp. dikenali sebagai salah satu penyebab kepada penyakit-penyakit bawaan air dan makanan. Kebiasaannya, manusia dan haiwan mengalami penyakit cirit-birit disebabkan parasit ini. Jangkitan *Cryptosporidium* dalam haiwan ternakan terutamanya lembu, kambing, biri-biri dan kerbau diterangkan di dalam kertas kajian ini termasuk ciri-ciri klinikal dan patologi dan interkasi di antara *Cryptosporidium* spp. dan enteropatogen yang lain. Maklumat mengenai penyebaran atau faktor-faktor epidemiologi dan kerugian ekonomi dalam kalangan haiwan ternakan yang berlainan turut diterangkan dalam kertas ini. Jangkitan *Cryptosporidium* merupakan satu masalah utama dalam kalangan ruminan kecil memandangkan jangkitan ini menyebabkan gejala neonatal cirit-birit pada haiwan ternakan. Jangkitan *Cryptosporidium* dalam ruminan kecil mungkin menjadi penyumbang kepada kriptosporidiosis dalam kalangan manusia. Memandangkan ubat-ubatan yang sedia ada tidak berkesan untuk merawat kriptosporidiosis, amalan pengurusan yang berkesan adalah wajib untuk menghalang kriptosporidiosis daripada merebak.

Kata kunci: Neonatal, *Cryptosporidium*, zoonosis, cirit-birit, haiwan ternakan

1.0 INTRODUCTION

Cryptosporidium is categorized under eukaryote in the phylum of Apicomplexa. All *Cryptosporidium* species are intracellular and obligate protozoa parasites. *Cryptosporidium* produces encysted stage of oocysts in the host faeces [1]. *Cryptosporidium* is small protozoan parasites with the size 4-6µm that inhabit microvillus border of epithelium mucosal in many vertebrates including human [2]. *Cryptosporidium* was named by a scientist, named Tyzzer in 1907. However, *Cryptosporidium* only acknowledged as one of the causes for gastrointestinal diseases in 1955 when it was found in turkeys with diarrheic infections. In the early 1980s, *Cryptosporidium* was abundantly found in some ruminants with diarrhea infections and it was assumed that *Cryptosporidium* as the primary caused for diarrhea diseases. Besides that, *Cryptosporidium* infection had been spread in humans and the infection mostly occurs among immunodeficient patient such as AIDS patients. The spreading of *Cryptosporidium* infection in humans had given more attention to the worldwide because this infection can lead to life-threatening disease in humans. In immunocompetent patients, *Cryptosporidium* can cause a short-term diarrheal disease within 3-20 days and the disease may cure spontaneously without any treatment. Meanwhile, in immunocompromised patients, *Cryptosporidium* infection may lead to prolonged, life-threatening, cholera-like illness. Currently, there is no effective therapy for *Cryptosporidium* infection. Reports of *Cryptosporidium* infections in the biliary tree and respiratory tract had suggested that *Cryptosporidium parvum* is not only infecting gastrointestinal tract, but it also infecting respiratory tract and biliary tree, especially in immunocompromised patients [3].

2.0 HISTORY OF *CRYPTOSPORIDIUM*

In 1895, J. Jackson Clarke claimed that he had found swarm spores known as encapsulated free coccidia in the cardiac glands of the stomach of the mouse. The mouse was sickened and killed on the seventh day after it had been given food material contained ripe spores of *C. falciforme*. The swarm spores were lying free on the mucous membrane surfaces and expanding in the ducts. Twelve years later, a well-known American parasitologist had named and described these swarm spores as *C. muris*. *C. muris* had infected gastric epithelium of some laboratory mice (English mice, *Mus musculus*, and Japanese waltzing mice) that had been used in Tyzzer's research program. *C. muris* was placed in a new genus because the oocysts of this parasite were not same with coccidia. The sporozoites of *Cryptosporidium* oocysts were not surrounded by any sporocysts [4]. *Cryptosporidium* was named because of the absence of sporocysts in the oocysts [5].

By using only a light microscope, Tyzzer was able to distinguish and describe the morphology and sequence of sexual and asexual life cycle stages of *Cryptosporidium* even though the stages are barely seen under a light microscope. Tyzzer had recognized the oocyst and the oocyst was sporulated while it still attached to the host cell and the attachment lead to autoinfection conditions. Even though Tyzzer just used a light microscope to observe the life cycle stages, Tyzzer concluded that *Cryptosporidium* obtained all nutrients from the host cell through organ attachment. It is called feeder organelle and totally parasitic in nature. In 1986, Tyzzer had used an electron microscope and done a slight amendment to the previous life cycle. The second generation of schizogony had been identified and this identification had led to the establishment of accepted life cycle model. Moreover, the used of electron microscopy and freeze fracture had proved that *Cryptosporidium* is intracellular parasites [5]. Three years later, Tyzzer had defined many stages in the life cycles of *C. muris* and in 1912 he had detailed more on the life cycle and morphology of the second species of *Cryptosporidium* (*C. parvum*), that found in the small intestine of mice. Seventeen years later, Tyzzer had discovered the developmental stages of another species of *Cryptosporidium* found in the caecal epithelium of chicken. This description was not detailed like the first description because he thought the species that are found in the chicken was *C. parvum*. *C. muris* and *C. parvum* were not considered as medically or economically important and these parasites received little attention from biomedical researchers [4].

Approximately 19 additional species of *Cryptosporidium* from reptiles, mammals, fishes, and birds had been identified resulted from the studies conducted from 1961 to 1986 on structural features of *Cryptosporidium* oocysts. However, only a few, including two originally species described by Tyzzer (*C. muris* and *C. parvum*) are considered valid. Even though Tyzzer had recognized *C. muris* in laboratory mice during 1907, *Cryptosporidium* only associated with morbidity and mortality in 1955 through a study conducted by Slavin [6]. The study showed that a few 10- to 14- day old turkey poults had experienced severe diarrhea and even death. These illnesses were caused by a new species of *Cryptosporidium*, *C. meleagridis*. *C. parvum* had raised the attention of veterinary medical profession when *C. parvum* had been associated with bovine diarrhea in 1971. Many case reports of *C. parvum* infections in different animals had been reported after this incident happened. *C. parvum* is acknowledged as an important source of neonatal diarrhea in lambs and calves. Another new species of *C. baileyi* is recognized as an important caused for respiratory disease in poultry. In 1976, the first cases of *Cryptosporidium* infection in human were reported and subsequent reports of *Cryptosporidium* had proved that *Cryptosporidium* may lead to a life-

threatening, prolonged, cholera-like illness in immunodeficient patients, especially AIDS patients, and a short-term diarrheal illness in immunocompetent patients [3].

3.0 TAXONOMY

Apicomplexa had been named and identified based on several factors such as a location of endogenous stages, host specificity, and morphology of exogenous or endogenous stages [7]. Researchers can identify numerous genera and morphologically different species based on different measurements. Therefore, the naming of new species depends on the oocyst structure or morphology. Unlike other different species, the morphology of *Cryptosporidium* oocyst is not reliable for identification of a new species. The similar size of oocysts can lead to confusion when identifying the *Cryptosporidium* species. Initially, same like most coccidia, *Cryptosporidium* was classified as a host-specific parasite, and the new species of *Cryptosporidium* were named based on the host where *Cryptosporidium* was found [7]. For example, *Cryptosporidium agni* was named when it is found in sheep faeces, and *Cryptosporidium garnhami* or *Cryptosporidium enteriditis* was named when it is found in human faeces, but since these *Cryptosporidium* species lacked taxonomic data that can distinguish these species clearly from other species (molecular data, biological, and morphological) made them become non-valid names. Unfortunately, some research revealed that *Cryptosporidium* can be transmitted to many hosts and some researchers suggested that *Cryptosporidium* is a single-species genus [8]. Besides that, the morphological differentiation of exogenous stage of *Cryptosporidium* oocysts was deficient to differentiate one species from another species except for the species with small oocysts or large oocysts. For example, *C. muris* is a parasite with large oocysts and it parasitize gastric mucosa of the mammals while *C. parvum* is a parasite with small oocysts that parasitize mammals' intestines [9].

Cryptosporidium had been identified in fish, mammals, reptiles and birds [10]. In mammals, *Cryptosporidium* had been identified in six orders of mammals which are *Perissodactyla, Primates, Rodentia, Artiodactyla, Lagomorpha,* and *Carnivora* [8]. *Cryptosporidium* species that considered valid species currently are *C. andersoni* (cattle), *C. parvum* (ruminants and humans), *C. hominis* (humans), *C. galli* (birds), *C. baileyi* (chicken and some other birds), *C. meleagridis* (birds and humans), *C. molnari* (fish), *C. canis* (dogs), *C. muris* (rodents and some other mammals), *C. felis* (cats), *C. saurophilum* (lizards and snakes), *C. serpentis* (snakes and lizards), and *C. wrairi* (guinea pigs) [10].

4.0 MORPHOLOGY AND LIFE CYCLE

Genus *Cryptosporidium* has the smallest size of oocysts compared to other coccidia. The oocysts are spherical to ovoid shape and fully sporulated averaged 5.6 µm for *C. muris* and 4.5 µm for *C. parvum*. Each sporulated oocysts of *Cryptosporidium* contain four sporozoites and a residuum consisted of an ovoid or spherical membrane-bound globule and numerous small granules. Other morphological characteristics that usually observed in coccidian oocysts such as polar granules and micropyle are not found in *Cryptosporidium* oocysts. The oocyst wall of *Cryptosporidium* is colourless and smooth with approximately 50 nm in thickness. The oocyst wall composed of two electron-dense layers that separated by a thin electron-lucent space. Sometimes, a faint line extends from partially one pole of the oocyst over the wall circumference, when it is seen under a light microscope [11].

The life cycle of parasite is usually direct, but unfortunately life cycle of coccidia is not always clear whether the sporulation of the oocysts take place within or outside the host. Until now, the infective stage in faeces was still in controversy among the researchers [12]. *Cryptosporidium* is the parasite that is identified as homoxenous (monoxenous) and *Cryptosporidium* is capable completing its life cycle in a single host. Compared to other coccidia, oocysts of *Cryptosporidium* do not need any sporulation outside of the host and the oocysts are excreted from the host's gut in a fully infective form. This will lead to a potential cause of direct faecal-oral transmission [13].

In 1912, Tyzzer had identified an oocyst containing four naked sporozoites in mouse faeces [12]. The developmental cycle of *Cryptosporidium* is completed in approximately 3 days (range, 1-8 days), and the organism develops through some stages which are excystment, asexual multiple budding (merogony or schizogony), sexual proliferation (gamogony), zygote and oocyst formation and sporulation (sporogony). The infection in a suitable host is triggered when the host ingested or inhaled the oocyst of the parasite which in the transmissible stage. Then, the oocyst will undergo excystment stage, and the oocysts releasing four motile sporozoites in the gastrointestinal tract of the host. The sporozoites are immediately infective and actively invasive to the host [13]. After sporulation, merogony and gametogony cycles will take place immediately within the host's body. The sporozoites infect the microvillus borders in enterocytes and resulting to the formation of trophozoites. The trophozoites differentiate into meronts that consisting eight merozoites. After that, gametogony phase is activated. In this phase, microgametes fuse with macrogametes and lead to the formation of specialised protective outer walls of zygotes. Lastly, the development of zygote into oocysts takes place [14]. As a result, thin-walled and autoinfective

oocysts are produced while the organism still attached to enterocytes [13].

There is a positive relationship between life cycle length with the host species, host's immunological status, and the age at which the host acquired the infection. Very young animals are at greater risk for *Cryptosporidium* infection, thus the relationship in inverse when it is related to age. In experimental animal studies, the life cycle of specific pathogen free can be as short as 72 hours in lambs infected since the first day of life. This period is a period considerably shorter than the life cycle of other enteric coccidia [14]. There are three factors that contribute to a very heavy infection and massive shedding of oocysts in the host's faeces which are lacking of a necessity for external maturation or for a second host, autoinfective oocysts production and multiple reutilizing the organism in asexual stage. For example, animal studies showed that infected calves can excrete up to 1,010 oocysts daily for up to 14 days during active infection, and infected humans also may shed a similar number of oocysts like in animal studies. The massive shedding of oocysts in animals and humans may lead to many problems in clinical management of patients individually and their contact. Besides that, there will also a difficulty in controlling the secondary spread of oocysts transmission through waterborne transmission [13].

5.0 *CRYPTOSPORIDIUM* INFECTION IN CATTLE

5.1 Historical Background

Cryptosporidium infections in cattle were firstly reported in cattle in the early 1970s [15, 16, 17]. The role of *Cryptosporidium* as primary enteropathogen was neglected when the infection is associated with other bacterial or viral enteropathogens. In 1980, Tzipori *et al*. had proved that the outbreak of neonatal diarrhea was caused by *Cryptosporidium* infection solely. In the following years, the new method to isolate infective oocysts had been developed and had proved that Cryptosporidium caused clinical diarrhea in calves [18]. *Cryptosporidium* infections in cattle had brought attention to public. Cross-transmission of *Cryptosporidium* infection to humans from cattle might happen when cattle grazing near the farms and sources of water [19].

Previously, only two species of *Cryptosporidium* can be identified in cattle which are *C. andersoni* (7.4 x 5.5 m, ellipsoidal oocysts), and *C. parvum* (5.0 × 4.5 m, spherical oocysts) [20]. Recently, several studies about *Cryptosporidium* infections in cattle in developed country have shown the presence of four *Cryptosporidium* species in dairy cattle which are *Cryptosporidium andersoni*, *Cryptosporidium parvum*, *Cryptosporidium ryanae,* and *Cryptosporidium bovis* [21]. A study done in Egypt showed that 57 pre-weaned calves infected with *Cryptosporidium* infections which are *C. parvum* (30/69), *C. ryanae* (13/69), *C. bovis* (7/69) and *C. andersoni* (7/69) [21].

C. parvum infects neonatal calves' intestines with high rates of infections. *C. parvum* infections usually accompanied with other diarrheal diseases. Meanwhile, *C. andersoni* infects adult and juvenile cattle's abomasum with lower rates of infections compared to *C. parvum* [22]. *C. parvum* is found infected calves less than 3 weeks of age [20]. *C. andersoni* were mostly infected cattle older than two months of age. The infection reduced production of milk in cattle [21, 23]. *C. parvum* genotype that infects cattle can also infect other mammals including human. There is no evidence to prove the zoonotic *C. andersoni* can infect other mammals except cattle [24].

C. bovis usually infects 2 to 11 months old dairy calves without the presence of overt disease. A deer-like genotype *Cryptosporidium* known as *Cryptosporidium ryanae* usually infects pre-weaned 2 to 11 months old calves without the presence of overt disease too [25]. Another *Cryptosporidium* species that infected cattle is *Cryptosporidium muris. Cryptospordium muris* usually infects abomasum of ruminants. A study done in British Columbia showed that 15% of the cattle were infected with *C. parvum* and 2% of the cattle were infected with *C. muris*. *C. parvum* was mostly infected calves aged 2 to 4 week and the number of faecal oocysts was significantly higher in calves aged 0 to 4 weeks. *Cryptosporidium* muris was only detected in calves more than 4 weeks of age. Lower prevalence of *C. muris* in calves compared to *C. parvum* had led to assumption that *C. muris* only infected older calves and adult cattle [26].

5.2 Clinical and Pathological Features

Prepatent and a patent period of kinetics of *C. parvum* oocyst shedding are experimentally ranging from 3-6 and 4-13 days, respectively. The excretion of the aoocysts are detected in calves as early as 3 days of age, which means that calves are immediately susceptible for *Cryptosporidium* infection shortly after or during birth. Calves that are raised in separation from *Cryptosporidium* are still susceptible to the infection at older age, but the infection is less severe. Most of the calves experience diarrhea starting from 3-5 days (during pre-incubation period) and last from 4 to 17 days [18]. *Cryptosporidium* spp. are obligatory and ubiquitous parasites that infect gastrointestinal epithelium of numerous range of vertebrates. The infection can lead to gastroenteritis and variable severities of diarrhea [21]. *Cryptosporidium* infections also influence the animal production rate as the infection can impair conversion of feed, adversely affect the growth of the animal, and reduce production of milk [27].

Besides that, *Cryptosporidium* also may negatively affect the health of young cattle by causing diarrhea infection which can lead to low productivity of

cattle. *Cryptosporidium* causes diarrhea in cattle-has resulted from the invasion of parasite and destruction of epithelial, subsequently lead to mild to moderate villus atrophy and microvillus shortening and destruction [18]. These problems lead to weakened transport and nutrient digestion, and finally, the death of the affected animals [24]. *Cryptosporidium* is usually infected heavily in the distal small intestine of the host, but the lesions exist in the colon and caecum and rarely in the duodenum. Pathological observations related to *Cryptosporidium* infections are mild to moderate villus atrophy, changes in surface epithelium, and villous fusion. Moreover, neutrophils and mononuclear cells was seen infiltrated lamina propria, a/b T cells, CD8+ and CD4+, and g/d T-cells were accumulated in intestinal villi after primary exposure to the infections, while the challenged immune animals show only an increase in CD8+ numbers [18].

5.3 Interaction with other Enteropathogens and Epidemiology

Cryptosporidium parvum usually infecting calves in the first week of life. The *Cryptosporidium* infection is usually accompanied with other bacterial, viral or other parasitic pathogens [19]. The most common bacterial, viral or other parasitic pathogens infecting calves together with *Cryptosporidium* are *Escherichia coli,* coronavirus, *Salmonella* and rotavirus. Most studies done in different countries showed that *Cryptosporidium* and rotavirus agents were mostly found in calves with diarrhea [28].

Diarrhea in calves under 3 days old is usually caused by enterotoxigenic *Escherichia coli* (ETEC), thermolabile and thermostable enterotoxins and colonization factors. Calves under 3 days old had a higher tendency to acquire diarrhea caused by ETEC because they had a diminished adherence of ETEC to enterocytes after a few days of life. For the calves between 4 days and 6 weeks of age, digestive problems are usually caused by *C. parvum* or other types of viruses such as coronavirus, rotavirus, and bovine viral diarrhea (BVD) virus [18]. A study in central Spain showed that the enteropathogens infected calves with *Cryptosporidium* infection were 27.8% for *E. coli*, 87% for rotavirus, 11.1% for coronavirus, and 1.8% for *Salmonella* [28]. A study done in Nigeria showed that *Cryptosporidium* infection was highest in calves between 7-12 months (28.1%), followed by calves that were less than 6 months of age (27.3%), while adult cattle had the least infection rate (19.9%). High *Cryptosporidium* infection in calves between 7-12 months might be due to the calves raised in confinement area. When calves reared in confinement area, the immunity levels of the calves were low and the oocysts were easy can be transmitted among the calves [19].

5.4 Transmission/Epidemiological Factors

There are five important characteristics that contribute to the spread and epidemiology of *Cryptosporidium* infection which are highly resistant of oocysts to chlorine and acid, small size of oocysts, low infectious dose of oocysts, zoonotic potential of oocysts (*C. parvum* genotype 1), and fully sporulated and immediately infectious after excreted [29]. Major contributors of *Cryptosporidium* infections are calves less than 2 months of age. Infected calves may excrete between 109 and 1,010 oocysts per gram in the faeces [30]. Calves release a large amount of oocysts in the faeces. These oocysts may contaminate drinking water, recreational water and fresh foods. Oocysts contamination had led to *Cryptosporidium* outbreaks affecting animals and humans [31]. *Cryptosporidium* oocysts can be transmitted through oral transmission such as direct contact with infected host or ingestion of contaminated food or water [21]. Besides that, contaminated pasture runoff also indicated as one of the sources for *Cryptosporidium* outbreak in humans [27].

Other potential risk factors are husbandry, management system, and age of the host. A study done in Egypt showed that, out of 96 calves, 29 (30.2%) of the investigated calves were infected with *Cryptosporidium* spp.. Out of 51 faecal samples from the first farm, 15 faecal samples were infected with *Cryptosporidium* oocysts. Out of 45 faecal samples from the second farm, 14 faecal samples were infected with *Cryptosporidium* oocysts. The differences between the results from two farms might be due to different breeds of calves, age of host, seasonal variation, management system, nursing conditions and sanitary conditions at the farm [30].

Another study done in Iran showed that, out of 480 cattle, 30 (6.25%) were infected with *Cryptosporidium* infection. The infection rates were significantly higher in cattle less than 6 months old of age (10.8%) than other age groups. The shape and size of oocysts (4.5–5.4 x 4.2–5.0 mm) found in this study were similar with *C. parvum*. High prevalence of *Cryptosporidium* infections in this age group might be due to poor immunity of newborn calves and easily infected with diseases [27].

5.5 Economic Losses

Diarrheal disease in neonatal calves causes serious economic losses. The economic losses happen due to cost of therapy, mortality and poor growth of the calves [27]. Cryptosporidial infection in neonatal calves give a greater impact to economic as the infection lead to diarrhea, growth retardation, dehydration, and mortality [18]. *C. parvum* infections in neonates especially during the first month of life can negatively affect the growth of neonates and lead to economic loss to the country [27]. Neonatal calves that infected with *Cryptosporidium* infections and suffered from diarrheal problems need special

care such as fluid therapy, hygienic measure, feeding of electrolyte solutions, and drug administrations. These therapies may lead to economic losses indirectly as the therapies are costly, time-consuming, and labour consuming [18].

6.0 *CRYPTOSPORIDIUM* INFECTION IN GOAT AND SHEEP

6.1 Historical Background

Cryptosporidium infection is a primary cause of neonatal diarrhea in domestic small ruminants such as sheep and goats. Sheep and goats represent as important sources of agricultural economy in most countries. *Cryptosporidium* infection in lambs and goat kids causes severe clinical symptoms and death to the animals. However, there is a lack of study done on the occurrence of *Cryptosporidium* infection in sheep and goats [32]. Cryptosporidial infection in lambs was firstly detected in Australia, but there was no actual causative agents detected in the study because the infection was coincidental infections with other pathogenic bacteria [16]. In the early 1980s, the role of *Cryptosporidium* as a primary agent causing diarrhea in lambs was proved through experimental and natural infections' studies [33, 34]. In the following years, many researchers had reported *Cryptosporidium* infection in lambs and those studies had indicated that C. *parvum* was a primary cause of diarrhea outbreaks in neonatal lambs and goat kids [35].

6.2 Clinical and Pathological Features

Cryptosporidium infection in small ruminants is more frequent during the first two weeks of life. As the age of infection increases, the severity of clinical symptoms decreases. The mean number of oocysts shed during the peak of infection may be approximately 2,109 oocysts and total output of oocysts can be more than 1,050 [36]. The prepatent period of *Cryptosporidium parvum* in small ruminants is approximately 4 days and the clinical symptoms were more severe in lambs and goat kids. Normally, animals between 1 to 5 weeks of age had a higher tendency to get infected with C. *parvum*, and the infected animals will demonstrate clinical signs between 5 days and 2 weeks. Oocyst excretion in infected lambs and goat kids is started on day 3-6 post-infection, lasting for 6-9 days, and persisting at asymptomatic but detectable level until 1 month of age [37]. Clinical cryptosporidiosis mostly occurs in goats >4 weeks old [37].

The symptoms of *Cryptosporidium* infection in lambs and goat kids from mild to severe diarrhea, and accompanied with other clinical symptoms such as depression, abdominal pain, and anorexia. The diarrhea is soft to liquid, yellow, and has a strong odour. There were a large number of oocysts (108 to 1,010 oocysts/g) detected in diarrheic stool [23]. Mortality is normally low in naturally reared suckling lambs, and the rate of mortality of infected animals increases when the disease is associated with insufficiencies in husbandry or nutrition, or the animals had concurrent infections [35].

Histologic lesions of infected animals demonstrated dilation of the crypt of Lieberkhiin, villous atrophy and fusion, and infiltration of lamina propria with mononuclear cells and neutrophil [38]. This pathology can lead to the establishment of clinical signs such as maldigestion, diarrheas due to malabsorption, and osmotic effects. When diarrhea lasting for 5-7 days, it may lead to abdominal tension, inappetence, lethargy, dehydration and death, even though the diarrhea is self-limiting disease [39]. A study done in Zaragoza, Spain showed that *Cryptosporidium* was found infecting extra-intestinal tissues of a two-month old lamb. The infected extra-intestinal tissues include gall bladder, mesenteric lymph nodes, uterus, and respiratory tract [40].

6.3 Interaction with other Enteropathogens and Epidemiology

Diarrhea disease in neonatal ruminants usually accompanied with infection from other enteropathogens such as *E. coli*, coronavirus, and rotavirus [37]. Diarrhea will cause the destruction of small intestine microvilli, and this condition is promoted with combine infections of *Cryptosporidium* spp. with rotaviruses, *E. coli*, *Salmonella* spp., and coronaviruses. The combination of *Cryptosporidium* infection with other enteric pathogens degenerates the prognosis and clinical signs and made the treatment become complicated. This situation leads to higher mortality and malnutrition to infected animals. Lambs may die within 2-3 days of the onset of diarrhea if the mixed infections were severe [39].

A study done in Italy showed that from 200 lambs in a farm located in the province of Grosseto (Tuscany, Central Italy), about 50% of the lambs were infected with *Cryptosporidium* infection and the mortality rate was close to 80%. The infected lambs demonstrated yellowish liquid, severe diarrhea, weight loss and depression. A post-mortem examination on dead lambs showed the lambs had liver congestion, pulmonary edema, and catarrhal enteritis. Samples from the lambs (gut, liver, lung, intracardiac cloth, and kidney) were tested for other pathogenic bacteria such as *E. coli*, *Clostridium perfringens*, coronavirus, and rotavirus, and the result was negative. Cryptosporidium oocysts were only detected in the lambs by using microscopic examination and confirmed with ELISA test [41].

6.4 Transmission/Epidemiological Features

Newborn animals that infected with *Cryptosporidium* can serve as asymptomatic carriers that transmit small numbers of oocysts into the environment [42].

Meanwhile, adult animals were considered having a strong immune response towards heavy infection and clinical disease of C. *parvum*. Asymptomatic adult sheep and goats may serve as healthy carriers and source of infections to young sheep and goats, especially during the periparturient period [23]. Besides that, transmission of *Cryptosporidium* spp. also through direct contact from contaminated food or waters [43]. The occurrences of *Cryptosporidium* spp. in animals also were dependent on extrinsic factors such as climate change and farm management and a variety of intrinsic factors such as age, nutritional status, and immune status of the host. The transmission of *Cryptosporidium* infection between or within the animals is usually through faecal-oral route by accidental ingestion of infective oocysts excreted by infected hosts [44]. Diarrheic lambs or goat kids can excrete as many as 4.8-109 oocysts per gram of faeces, and they can act as a potential source of infection to humans by contaminating the water catchment areas [45].

Different *Cryptosporidium* prevalence in sheep and goats from different geographical regions might be attributed from the different infectivity of *Cryptosporidium* spp. or differences in contamination of the environment with oocysts of parasite. Besides that, different quality of hygienic conditions in husbandry systems and different grazing practices also contributed to the different prevalence of *Cryptosporidium* infections in small ruminants [32].

It was found that different management system may contribute to infection of small ruminants with *C. scrofarum, C. andersoni,* and *C. xiaoi.* The small ruminants usually graze freely in grasses and shrubs along the road sides, gardens, near homes and they share the feeding grounds with other livestock such as cattle and pigs. This may lead to cross-transmission of *Cryptosporidium* species from cattle or pigs to the small ruminants [46].

Other factors that affect the infection rates of *Cryptosporidium* in small ruminants are age and hygiene condition of management systems. The usage of straw in mangers and periodic changing of the straws had successfully reduced the risk of *Cryptosporidium* infection in small ruminants [47]. A study done in Brazil showed that, out of 105 faecal samples, only 4.8% contained oocysts of *Cryptosporidium*. All positive faecal samples were from juvenile goats with the age range from 15 days to 3 months. No oocyst was detected in adult goat samples. The positive prevalence of *Cryptosporidium* infection in faecal samples might be due to the poor hygienic condition of management system. The farm was not frequently cleaned. The accumulations of foods and droppings on the floor had attracted flies. The flies may act as mechanical vectors that transmitting oocysts and contaminating foods and waters for ruminants [47].

A study done in Zaragoza demonstrated that 344 lambs (59%) were positively infected with C. *parvum*. The infection rates of *Cryptosporidium* were significantly higher in lambs aged 1 to 21 days (66.4%), than in lambs aged 22 to 90 days (23%). The study showed that age was strongly related with shedding odds of C. *parvum* oocysts [35]. Another study done in Greece found that 75% of the lambs with age group <14 days were infected with *Cryptosporidium* spp. while only 7.89% of the lambs with age group 15 to 30 days old were infected with *Cryptosporidium* spp.. The study proposed that *Cryptosporidium* infection is more frequent occurred during the first 2 weeks of life and when the age at infection increases, the severity of symptoms decreases [39].

6.5 Economic Losses

Cryptosporidium infection in small ruminants possessed an important economic impact the farmers because the infection lead to high morbidity and sometimes high mortality rates to infected animals [48]. *Cryptosporidium* infection also gives a greater impact on the economy as the infection leads to impaired feed conversion and negative effect on the growth [49]. *Cryptosporidium* infection caused diarrhea and also death to the animals and these problems lead to economic losses. Young animals are more susceptible to the infection compared to adults and adults may serve as a healthy carrier and a source of infection to the young animals [23]. Besides, *Cryptosporidium* infection can lead to mortality of the animals, retarded the growth of infected animals, increase the cost of drugs and veterinary assistance, as well as increase the staff labour [39].

A study done in Brazil showed that the infected animals died within a week after diarrhea started. Even the animals had been treated with gentamycin and oral and intravenous fluid therapy, the animals were not cured from the infection. Faecal smears of dead animals showed that the samples contained large numbers of *Cryptosporidium* oocysts. This study proved that *Cryptosporidium* infections gave negative impact to the economy due to the mortality of the infected animals and waste in drug cost [50].

7.0 *CRYPTOSPORIDIUM* INFECTION IN BUFFALO

7.1 Historical Background

Cryptosporidium oocysts were firstly detected in zebu cattle and buffaloes in India during 1992 [51]. There were two types of *Cryptosporidium* species that commonly infecting cattle and buffaloes which are *Cryptosporidium parvum* and *Cryptosporidium andersoni*. A study in Bangalore, India showed that out of 21 positive calves, 10.8% were infected with *C. parvum* and 5.9% were infected with *C. andersoni*. The species of the *Cryptosporidium* were identified based on the micrometry and morphology of the

oocysts. *Cryptosporidium parvum* oocyst is spherical in shape and usually found in young calves. Meanwhile, *C. andersoni* oocyst is oval in shape and commonly found in heifers and adult milking animals [52]. C. *parvum* usually infects epithelial lining of small intestines of pre-weaned calves while *C. andersoni* usually infecting abomasum of post-weaned calves and adult cattle. C. *parvum* caused diarrhea infection in cattle. Meanwhile, *C. andersoni* reduced milk production of the infected cattle [53].

Another study done in Egypt showed that from 17 positive water buffalo calves, approximately 59% were infected with *C. ryanae*, and 41% were infected with C. *parvum*. None of the adult buffaloes were positive with *Cryptosporidium* oocysts [54]. A study done in Spain demonstrated that, out of 347 faecal samples from buffaloes, 51 samples were positively infected with C. *parvum* oocysts only [55].

7.2 Clinical and Pathological Features

Cryptosporidium inhabits respiratory and intestinal surface epithelium of numerous species of mammals including birds, humans, fish, and amphibians [53]. *Cryptosporidium* infections lead to significant mortality and morbidity due to severe diarrheal disease [52]. *Cryptosporidium* spp. is Apicomplexan parasites that infect the gastrointestinal and respiratory tract of animals and humans [7]. Infected animals suffered from mild to severe diarrhea and severe respiratory disturbances. These problems negatively affect the production of animals [56]. Calves infected with *Cryptosporidium* spp. are usually asymptomatic. Only stress calves exhibit cryptosporidiosis symptoms [57]. Clinical symptoms of cryptosporidiosis are dehydration, inappetence, fever, dullness, and diarrhea together with mucus. Infected calves usually not respond to any treatment especially antibiotic therapy, and in severe cases, cardiovascular collapse and dehydration in infected animals can lead to mortality. Duration of diarrhea in neonatal calves varied from 2 to 23 days together with mild depression, fever, dehydration, and variable degree of anorexia. Diarrhea in calves above one month of age was self-limiting. Affected calves also showed growth rate reduction. *Cryptosporidium* infections destroyed microvilli of peptic glands and this lead to increase the quantity of plasma pepsinogen in infected animals [53].

A study done in Philippines showed that out of 38 calves, only one calf infected with *Cryptosporidium* on day 8. The results showed that calves produced 26.31% solid formed stools, 63.15% mushy stools and 10.5% watery stools on day 4. On day 8, the percentage of solid formed stools was decreased to 18.42%, the percentage of mushy stools was increased to 76.3%, and the watery stools was decreased to 5.3%. Meanwhile, the infected female calf had watery diarrhea in grayish colour with blood tinge and plenty of mucus. The diarrheic stool showed the typical signs of catarrhal enteritis. On day 12, the infected calf showed the same diarrheic stool consistency. A watery stool consistency had proved that the calf was infected with cryptosporidiosis [58].

7.3 Interaction with other Enteropathogens and Epidemiology

Neonatal ruminants with compromise or less developed immune systems are more susceptible to severe *Cryptosporidium* infection than healthy adult animals. Cryptosporidial is more lethal and severe when associated with other enteropathogens infections such as *E. coli*, coronavirus, rotavirus and *Salmonella* [58]. A study done in Brazil showed that out of 48 diarrheic faecal samples from buffalo calves, 29 were infected with *E. coli* and 4 were infected with *C. parvum* [59].

7.4 Transmission/Epidemiological Features

Cryptosporidium infection was transmitted through faecal oral transmission. The infective oocysts were transmitted through food, water or direct contact [60]. The significant source for *C. parvum* of cattle genotype is originated from humans and ruminants while the significant source of *C. hominis* is from humans only. The cattle genotype of *C. parvum* also can be found in other mammals such as cattle, sheep and human. Infected infants of buffaloes can shed a very high number of oocysts leading to environmental contamination [58]. Besides that, affected animals that undergo recovery period may become carriers and serve as a source of infection to transmit the infection to susceptible individuals [53].

Age factor contributes to a high prevalence of *Cryptosporidium* infection in buffaloes. A study done in Egypt showed that the highest infection rate was in animals aged from 1 to 30 days (62%) followed by 52% prevalence in animals aged from 1 to 2 months. The percentage of *Cryptosporidium* infection in buffalo calves aged 2 to 6 months were 49% and the lowest infection rates were demonstrated in calves aged more than 6 months (46%) [61]. Another study done in India showed that, out of 138 samples, the infection rates of C. *parvum* was the highest in calves less than 15 days of age (45.1%), followed by calves of 16 to 30 days of age (36.6%), and 31 to 45 days of age (35.9%). The study indicated that asymptomatic calves (non-diarrheic) act as a source of oocyst transmission to neonatal calves [62].

7.5 Economic Losses

Cryptosporidium is a primary enteropathogen causing neonatal diarrhea in calves and this problem lead to extensive economic loss to animal husbandry [62]. *Cryptosporidium* infection is a major cause of mortality and morbidity in young animals and immunocompromised patients. Cryptosporidiosis leads to economic losses due to the disease can cause mortality to the animals, retard the growth of animals and drugs cost [52]. Infected animals suffered from malabsorption and dehydration and

this led to severe economic losses to the country. Prolonged illness in infected animals lead to decrease in productivity, increase veterinary costs for drugs and veterinary aid and increase labour [53].

8.0 TREATMENT AND CONTROL OF *CRYPTOSPORIDIUM* INFECTION

Cryptosporidium infection is hard to control because there is no vaccine or drug found effective against the disease. A lot of chemotherapeutic trials had been done to resolve this disease but there is no successful treatment been identified. Hence, an effective ways in controlling this disease is by practicing effective management practices, sanitation and hygiene of the premises with proper diagnostic tools. Proper preventive measures can prevent cryptosporidiosis from happen. *Cryptosporidium* oocysts can be diminished by using 5% ammonia solutions together with heat on surfaces of premises. Besides that, the other ways to prevent this infection are by isolating the infected animals and ensuring the newborn calves received enough colostrum intakes [53]. In field conditions, passively acquires antibodies do not protect lambs and calves against cryptosporidiosis. Hyperimmune colostrum from immunized mother had shown less severe diarrhea and fewer released of oocysts compared with calves were fed with non-hyperimmune colostrum [53].

To date, drugs that partially effective for treatment and prophylaxis of cryptosporidiosis in ruminants are paromomycin, halofuginone lactate, and decoquinate [18]. Paromomycin is an aminoglycoside antibiotic and suitable to control *C. parvum* infections in man, animals, and cell culture models. Even though this drug is poorly absorbed from epithelium gut, this drug can be absorbed in small quantity across limiting apical membrane that bounded to the extracytoplasmic parasite. Paromomycin also can reduce the number of oocysts shedding and duration of diarrhea infections. However, this drug is not usually used for treating large ruminants [63].

Halofuginone lactate is a synthetic product of quinazolinone group with antiprotozoal activity. Halofuginone had shown effective role as antiprotozoal against *C. parvum* in cell culture model. In rat models, halofuginone had reduced the severity of cryptosporidiosis in caecum and small intestine but this drug failed to reduce the infection in the colon. It has been reported that halofuginone treatment also can prevent mortality and decrease the severity of cryptosporidiosis in calves. After the withdrawal of drug, no oocysts was detected for 7 days and this indicated that drug does not interrupt the life cycle of parasite but prevents re-infection of sprozoites or first generation of merozoites in the gut [63].

Decoquinate is a quinolone coccidiostat drug. This drug had shown a little effect against *C. parvum in vivo* and *in vitro* in suckling mice. Administration of decoquinate twice a day for 21 days to pregnant goats and goat kids had reduced diarrhea in natural and experimental infections. Decoquinate also had increased average daily weight gain of calves [63]. However, the use of decoquinate is still restricted in many countries due to the commercial availability of this drug [18].

9.0 CONCLUSION

Cryptosporidium infection is a major problem, especially in neonatal ruminants. *C. parvum* is the main enteropathogen found in first weeks of life of goat kids, lambs, and calves. *C. parvum* caused neonatal diarrhea syndrome in goat kids, lambs, and calves. Economic losses caused by this parasite are mortality, morbidity, decreased in productivity, increased veterinary costs for drugs and veterinary aid and increased labour. Since there is a lack of effective drug available to treat cryptosporidiosis, application of effective management practices, sanitation and hygiene of the premises is needed to prevent the spread of cryptosporidiosis.

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