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RESEARCH ARTICLE

Occurrence of caprine *Cryptosporidium* infection and its associated factors in goats from Pyinmana Township

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Abstract

A cross-sectional study was conducted to find out the prevalence of caprine cryptosporidiosis and its potential associated factors in Pyinmana Township. For this purpose, 306 faecal samples of goat from five quarters/villages were collected and examined by using modified Ziehl-Neelsen (Z-N) staining method and sugar floatation method. Kappa value was calculated to determine agreement between the two diagnostic tests. The kappa value showed substantial agreement between Z-N staining method and sugar floatation test. Associated factors were analyzed by Pearson Chi-square test based on the prevalence results diagnosed by sugar floatation method. Among 306 faecal samples, 135(44.12%) were positive for *Cryptosporidium* species infection. Among positive samples, 39.22% of the samples showed mild infection with oocyst density of ≤5 which was scored as +1. In univariate analysis, age, sex, breed, grazing, water source and sanitation were observed as having association ($P < 0.05$) with the presence of infection while mixed raising with other livestock was not associated.

Keywords:

caprine cryptosporidiosis, Ziel-Neelsen staining method, flotation method, associated factors

1. Introduction

The genus *Cryptosporidium* is composed of protozoan parasites that infect epithelial cells in the microvillus border of the gastrointestinal tract of all classes of vertebrates. They are found worldwide (Fayer and Xiao, 2007). The parasite was first described in mice in 1907 (Tyzzer, 1907). The first case of human cryptosporidiosis, which involved a 3-year old girl from rural Tennessee who suffered severe gastroenteritis for two weeks (Nime et al., 1976) and the first case of infection in goats was described in Australia in a 2 week-old-kid with diarrhoea (Mason et al., 1981). *Cryptosporidium* become an interesting emerging infection owing to its opportunistic behaviour in acquired immunodeficiency syndrome patients (Petersen, 1992) and children (Valentiner-Branth et al., 2003).

Cryptosporidium spp. infection can be acquired to humans by ingestion of oocysts that were excreted in the faeces of infected individuals. Transmission can occur from person-to-person, from animal-to-person, animal-to-animal, by ingestion of contaminated water and food or by contact with contaminated surfaces (Ramirez et al., 2004). Asymptomatic infection is common, children less than one year found to be infected in developing countries. In developed countries, the infection is most common in toddlers (Caccio and Pozio, 2006). *Cryptosporidium* infections in early childhood have been reported to be associated with subsequent impairment in growth, physical fitness and cognitive function (Guerrant et al., 1999).

Caprine cryptosporidiosis was first described by Mason et al. (1981). Lange et al. (2013) described that infected goat kids were considered as potential zoonotic reservoirs for human infections. Cryptosporidiosis in goats is a well-known disease which causes neonatal kid diarrhoea and incurs significant production loss to the goat husbandry (Xiao et al., 2010).

In Myanmar, cryptosporidiosis has not yet been studied in goats. Goats may serve as reservoirs of *Cryptosporidium* for human infections. Cryptosporidiosis has been associated with the risk of infection in humans through direct or indirect contact with infected animals. *Cryptosporidium* infection in goats should be considered a potential source of zoonotic parasites (Lange et al., 2013). Pyinmana Township is one of the townships located in Nay Pyi Taw area. Estimated goat population in Pyinmana Township is 1,502 (Source: 2017 animal census, LBVD, Pyinmana Township). However, the information on prevalence of cryptosporidiosis in goats from Pyinmana Township is lacking. Therefore, it is necessary to investigate the occurrence because of not only an important disease for farm animals but also a wide spread zoonotic disease for public. Furthermore, it is important to reduce economic losses faced by farm owners due to this disease. Therefore, this study is carried out to investigate the prevalence of *Cryptosporidium* infection in goats from Pyinmana Township and to identify factors associated with *Cryptosporidium* infection in goats from Pyinmana Township.

2. Materials and methods

A cross-sectional study on prevalence of *Cryptosporidium* infection in goats was carried out in Pyinmana Township from April 2017 to September 2017. Faecal samples were collected from goat farms within Pyinmana Township where located in the center of Nay Pyi Taw Union Territory. Five quarters/villages were selected randomly according to greater number of goat population as compared to other villages in the township. A total of 306 faecal samples were collected and examined for the presence of *Cryptosporidium* oocyst. About 25g of faecal sample was collected from the rectum or from the ground immediately after defecation. Separate disposable gloves and zip-locked plastic bags were

used for sample collection. Then the bags were labelled with animal individual numbers and stored in an icebox until examined. All samples were carried to the Department of Pharmacology and Parasitology, University of Veterinary Science, Yezin, Nay Pyi Taw and kept in the refrigerator. The identification of *Cryptosporidium* oocysts was performed by following standard laboratory procedures. For the detection of oocysts, the faecal samples were mixed with sugar solution and centrifuged for three times at 1500rpm for 10 minutes, and then supernatant fluid was discarded. The detection of oocysts in sediment was performed by using flotation method and modified Ziehl-Neelsen (Z-N) acid fast staining method. For flotation method, the sugar solution was poured into the test tube with sediment until full, the cover slip was put onto the test tube and waited for 10-15 minutes. After that, the cover slip was kept over the glass slide, and then oocysts were searched under the microscope (Zajac and Conboy, 2012).

For modified Z-N acid fast staining method, the identification of *Cryptosporidium* oocysts was conducted according to Clarke and McIntyre (2001). Thin faecal smear were left to the air to dry and fixed it in methanol for 2–3 minutes. The smear was stained with cold carbol–fuchsin for 5–10 minutes and differentiated in 1% hydrochloric acid–ethanol until colour ceases to flood out. It was rinsed in tap–water and counter stained with 0.25% malachite green (or methylene blue) for 30 seconds. Then, it was rinsed in tap–water again, blotted or drained dry. The stained smear was examined by high power objective (40×10) and confirmed the morphology using oil emersion. *Cryptosporidium* oocysts appeared as bright rose–pink spherical on a pale green background.

Oocyst density of each sample was determined according to the oocyst scoring system of Dagnall Teaching Laboratory, Liverpool School Tropical Medicine (1998), as follows: rare (+) for less than or equal 5 oocysts per slide; few to moderate (++) for 5-10 oocysts per field of view; and numerous (+++) for 11 or more oocysts per field of view.

Calculation of Kappa value was performed and the agreement between the two diagnostic tests was interpreted. For Kappa value, Landis and Koch (1977) stated that <0.0 is poor agreement, 0.0 – 0.2 is slight, 0.2 – 0.4 is fair, 0.4 – 0.6 is moderate, 0.6 – 0.8 is substantial and 0.8 – 1 is excellent.

Data files of questionnaires and laboratory results were entered into Microsoft Excel Sheet. Pearson Chi-square test was used to identify associated factors of caprine cryptosporidiosis by using SPSS version 17 at $P < 0.05$ level of significance.

3. Results

The prevalence of *Cryptosporidium* spp. infection in different study locations based on sugar flotation method is described in Table 1.

Table 1. Finding of *Cryptosporidium* spp. infection in study locations

No.	Quarter/Village	No. examined	No. (%) of positive animals
1	Katoesate	48	19 (39.6)
2	Nyaung Pin Thar	96	41(42.7)
3	Ahlyin Lo	67	30 (44.8)
4	OoKyae	49	25 (51.0)
5	YwarKout	46	20 (43.4)
	Total	306	135

Table 2. Distribution and analysis of associated factors

No.	Risk factors	No. of examined	No. of positive	Prevalence (%)	OR	95% CI	χ^2	P value
1	Age							
	≤7m	169	93	55.03	2.77	0.23-0.58	18.23	0.000***
	>7m	137	42	30.66				
2	Sex							
	Male	121	63	52.07	1.7	0.37-0.93	5.13	0.024*
	Female	185	72	38.92				
3	Breed							
	Htein San	228	122	53.51	5.75	0.09-0.33	31.99	0.000***
	Jade Ni	78	13	16.67				
4	Grazing							
	Yes	217	109	50.23	2.45	0.24-0.69	11.31	0.001***
	No	89	26	29.21				
5	Water Source							
	Common	185	92	49.73	1.80	0.35-0.89	5.98	0.014*
	Well	121	43	35.54				
6	Sanitation							
	1 time/day	202	103	50.99	2.34	0.26-0.70	11.39	0.001***
	2 times/day	104	32	30.77				
7	Mixed raising with other live-stock							
	Yes	59	27	45.76	1.09	0.52-1.63	0.080	0.777
	No	247	108	43.72				

***= very highly significant at 0.001 level

* = significant at 0.05 level

3.1 Prevalence of *Cryptosporidium* spp. infection

Out of 306 faecal samples examined by modified Z-N staining method, 141 samples (46.08%) showed *Cryptosporidium* infection positive. When 306 faecal samples were examined by sugar floatation method, 135 samples (44.12%) showed Cryptosporidium infection positive.

3.2 Kappa agreement between two diagnostic tests

When Kappa value between the two diagnostic tests was calculated, the value for Kappa was 0.65. So it can be interpreted that the agreement between the two diagnostic tests is substantial.

3.3 Oocyst density of *Cryptosporidium* spp. by sugar floatation

Oocyst density of *Cryptosporidium* spp. in the study area was observed as 39.22% (120/135) for + and 4.90% (15/135) for + +. There was no samples showing + + +.

3.4 Prevalence of *Cryptosporidium* spp. infection in goat from different study locations

Among the different study locations, the prevalence of *Cryptosporidium* spp. infection in goat based on findings of sugar floatation method was found numerically highest in OoKyae village and the lowest infection rate was noted in Katoesate village.

3.5 Univariate analysis of associated factors

Hypothesized factors were examined by Pearson Chi-square test. The prevalence percentage included in the analysis was taken from the result of sugar floatation method. Factors of age, sex, breed, grazing, water source, and sanitation were noted as having association with *Cryptosporidium* spp. infection (Table 2).

4. Discussion

Cryptosporidiosis in goats is a well-known disease which causes neonatal kid diarrhoea and incurs significant production loss to the goat husbandry. The agents responsible for the disease in goats are *Cryptosporidium parvum* (*C. parvum*), *C. hominis,* and *C. xiaoi;* however, *C. bovis* has also been reported from goats and apparently there is report of a *Cryptosporidium* goat genotype (Xiao et al., 2010). A serious constraint to economical and intensive goat production is the mortality of kids as a result of diarrhoea (15–40%) up to the age of three months. Among the various diarrhoeal pathogens of goats from viruses, bacteria and parasites, *Cryptosporidium* spp. is the one principally involved (Noordeen et al., 2000; Ershaduzzaman et al., 2007).

Once thought to be a parasite of veterinary importance, *Cryptosporidium* has emerged as an important human pathogen, especially in pediatric, geriatric and immunocompromised patients where a fulminating infection may be life threatening (Fayer, 2004). *Cryptosporidium* becomes an interesting emerging infection owing to its opportunistic behaviour in AIDS patients (Peterson, 1992). Prevention depends on appropriate hygiene and proper water management and treatment (Collinet-Adler and Ward, 2010).

In Myanmar, although studies on bovine cryptosporidiosis have been carried out in some regions, studies on occurrence of it in caprine was not yet performed. Finding of this study was first report on prevalence of *Cryptosporidium* infection in goat in Myanmar. In this study, faecal samples of goats were collected from five villages/ quarters within Pyinmana Township and examined by microscopy using Z-N staining method and sugar floatation method.

The prevalence of *Cryptosporidium* infection in goats in Pyinmana Township was observed as 46.08% by Z-N test and 44.12% by sugar floatation test. It was much higher when compared to China (11.4%) (Rongsheng et al., 2014), Iran (18.86%) (Khezri and Khezi, 2013), Spain (20%) (Castro-hermida et al., 2005), Iraq (31.5%) (Alkhaled and Hamad, 2016) and Serbia (31.8%) (Zorana et al., 2006). However, the prevalence observed in this study was not much different with that of Korea (42.9%) (Park et al., 2006). The differences are likely due to different detection methods employed. Moreover, it also might be due to geographic variation,

different in climatic conditions, breed of goats and management practices. Zhang et al. (2013) reported that geographical and seasonal differences can also affect the occurrence of *Cryptosporidium* spp. infection.

Moreover, the prevalence in goat found in the present study was not so different in bovine, 57.3% in Mandalay city investigated by Lay et al. (2008) and 56% in Pyawbwe and Yamethin Townships by Bawm et al. (2014). Therefore, it could be assumed that the parasite is widely distributed in the environment. It is important for public health due to shedding of oocysts in the environment by infected hosts and increased risk of getting infection in human by contaminated water.

In this study, two diagnostic techniques, Z-N method and sugar floatation method were conducted. According to Casemore (1991) and Rekha et al. (2016), sugar floatation technique was found to be more specific and sensitive method than Z-N method by eliminating confused faecal debris and increasing the chance of finding protozoa oocyst, especially in asymptomatic individuals with low parasite discharge. Moreover, the kappa value showed substantial agreement between Z-N test and sugar floatation test. Therefore, the results obtained by sugar floatation test were considered to account as the prevalence rate of this study. The numerically highest prevalence of *Cryptosporidium* spp. infection (51%) was recorded in OoKyae village and the lowest prevalence (39.6%) was recorded in Katoesate village. It might be due to the poor sanitary condition of backyard farms and overcrowded housing in OoKyae village. Tiranti et al. (2011) also described that high prevalence of *Cryptosporidium* infection occurred in herds of poor drained area.

Among positive samples (44.12%) observed in this study, most of the samples (39.22%) showed mild infection with oocyst density of ≤5 which was scored as +1. It was clear that the infected goat act as a healthy carrier and it could be a source of infection for other animals. In the present study, most of all positive animals showed normal clinical appearance therefore it could be noted that those animals might act as reservoirs and discharged oocyst into the environment to infect other animals. According to Pereira et al. (2002), a single oocyst is sufficient to produce infection and disease in susceptible hosts.

Lange et al. (2013) described that infected goat kids were considered as potential zoonotic reservoirs for human infections. Based on the prevalence of *Cryptosporidium* infection in young animals observed in this study (55.03%), it can be assumed that goat kids might be important source of zoonotic transmission for infection. In this finding, most of the sampled goats acted as healthy carriers for shedding of oocysts. According to Geurden et al. (2008), goat kids are susceptible to infection than adult goats. Moreover Zhang et al. (2013) also mentioned that the prevalence of *Cryptosporidium* declined with the increasing ages of animals although hosts of all ages are affected.

This study identified that there was association between the presence of infection and age of goat. According to Pearson Chi-square test, the younger kids (≤7m old) had 2 times more likelihood to be infected than older goats (>7m old). Paul et al. (2014) reported that *Cryptosporidium* is regarded as one of the major enteric pathogen in goat kids and morbidity could be high in outbreaks of cryptosporidiosis in kids. Thus, veterinarians and goat farmers should notify the zoonotic transmission risk of *Cryptosporidium* from goat kids. Reports of zoonotic transmission of *Cryptosporidium* from goat kids to humans have been described by Lange *et al.* (2013) and Paul et al. (2014). Therefore, the finding of age as a related factor in this study might be due to the higher susceptibility of goat kids to *Cryptosporidium* infection than adults.

In the current study, breed showed association with the presence of infection. According to Pearson Chi

-square test, Htain San breed had 3 times more odds to be infected than Jade Ni breed. There was very limited information regarding the influence of goat breed on the occurrence of cryptosporidiosis. It could be considered that different occurrence of infection in Htein San and Jade Ni breed might be due to genetically variation.

In the present study, sex was noted as having association ($P < 0.05$) with the presence of infection. Male goat showed higher positive percent (52.07%) than female goat (38.92%). The male had about 2 times more likely to be infected than female. This might be due to more superior activity of male than female. Maikai et al. (2009) stated that males are more likely than females to disperse to other places. This condition might favour the male goats more likely to be infected than female goats.

This study showed that there was association between the presence of infection and water source. According to Pearson Chi-square test, goats drinking common water had 1.8 times more likelihood to be infected than goats drinking well water. And also, this study showed the association between grazing habit and presence of infection. Grazing goats had 2.5times more probability to get infection than non-grazing goats. Grazing animals and common water source were probably associated with the infection as grazing animals would drink the common water. Non-grazing animals drank the water from well so it had probably minimized chance to get infection. Infected hosts might excrete very high numbers of the transmission stages, whilst relatively few are necessary in order to initiate an infection, thus lending themselves to transmission via environmental contamination (Robertson, 2009). There were many investigations showing evidence of high risk of contamination in common water near grazing area (Yoder and Beach, 2010). Therefore, it should be notified sharing of common water by humans and animals might favour the getting of infection.

In the present study, there was having association with number of sanitation per day and presence of infection. Goats from farms where sanitation was conducted once a day had 2.34 times more likelihood than goats from farms conducting sanitation 2 times per day. Mohammed et al. (1999) described that daily disposal, cleaning of bedding and moving of the animals after weaning were significantly associated with the decreased risk of infection. On contrary, the potential for environmental contamination depends upon a variety of factors, including the number of infected non-human hosts, the number of transmission stages excreted, agricultural practices, host behaviour, geographic distribution, sanitation, safety of drinking water and food sources and supplies, and the climate and hydrogeology of the area (Slifko et al., 2000). Paul et al. (2014) reported that effective control of the disease is probably possible with the knowledge of epidemiology of the disease coupled with the use of appropriate sound sanitary and management practices. Scorza and Tangtrongsup (2010) also stated that good sanitation practice is important in crowded area to reduce the infection rate. Fayer and U -ngar (1986) indicated that poor sanitation may have had a role in the outbreak since the premises were thought to be contaminated with large numbers of oocysts from an artificially reared kid. Therefore, it could be suggested that sanitation was important for the prevention of disease and at least 2 or more times a day should be sanitized in the farm and should practice cleaning of hands after contact with goats and goat manure.

According to Pearson-Chi square analysis, there was no association with mixed raising with other livestock in the farm. In this study, most of the farm (80.71%) practised raising only goats whereas no association with the presence of infection. According to Khezi and Khezi (2013), overcrowding the lambs and

goat kids together in a small area caused high infection rates. Those authors also indicated that the quality of hygienic conditions of animal husbandry and grazing practices may have influenced the exposure of animals to cryptosporidial infection. However, no association observed in this study might be due to grazing of goats near the common water source whether farm owner practiced mixed raising with other livestock or not.

According to this study, continued researches are needed to improve knowledge of the parasite's epidemiology, biology and taxonomy. Molecular diversity also needed as improved detection protocols are capable of differentiating species and genotypes. *Cryptosporidium* infection in goats has been reported all over the world in the past ten year. However, most of these studies used microscopic examinations, immunofluorescence tests, or modified Z-N staining techniques. Few reports used molecular methods, and the features of *Cryptosporidium* species/ genotypes were still unclear (Rongsheng et al., 2014). The transmission dynamics and the risky extent of zoonotic transmission of *Cryptosporidium* spp. need to be elucidated with sufficient molecular epidemiologic data from humans, animals and water source in the future.

According to the present study, it could be assumed that cryptosporidiosis is a well-documented disease in livestock, affecting mostly neonates and the threat to human health was major concerns due to shedding of large number of oocysts. According to Budu -Amoako et al. (2011), young animals are shedding large number of oocysts. Prevention and control measures need to be adopted and regulated in the animal environment not only for animal health but also for human health.

This study also underlines the need to improve surveillance networks in order to get a better understanding of the health status and the epidemiological patterns of the *Cryptosporidium* spp. infection in South-East Asia. This information is also necessary to allocate resources according to the real burden of the various diseases and the socioeconomic contexts of the countries. To be efficient, these approaches have to be undertaken within the frame work of the One-Health concept, taking into account all the components of the diseases at the human–animal–ecosystems interface. Further investigation is needed in order to assess the distribution in all livestock with larger sample size. In addition, molecular identification of *Cryptosporidium* spp. and assessment of its risk relevant with human infection are also necessary.

The present finding is the first investigation on the microscopic prevalence of caprine cryptosporidiosis in Pyinmana Township, Nay Pyi Taw area, Myanmar and pointed out kids being at high risk of infection than older ones. Awareness on prevalence and factors associated with *Cryptosporidium* oocyst shedding by goat might be helpful in designing prevention strategies to minimize the impact on caprine industry due to this parasite and potential hazards to public health.

5. Conclusions

According to the present study, it was concluded that the overall prevalence of caprine cryptosporidiosis in Pyinmana Township was 44.12% (135/306) diagnosed by sugar floatation method and 46.08% (141/306) diagnosed by Z-N staining method. The numerically highest prevalence of *Cryptosporidium* infection (51%) was recorded in OoKyae village whereas the lowest occurrence (39.6%) was noted in Katoesate village. Among 135 goats showing positive result, (39.22%) shed *Cryptosporidium* oocyst with rare rate (+) while 4.9% of goats shed moderately (+ +). In univariate analysis, age, gender, breed, grazing, water source and sanitation were noted as having association while mixed raising with other livestock was not associated with the presence of infection. Younger goats (≤7mont-

-hs) had 2 times higher probability to get infection than older goats (>7months). Htein San breed had 3 times greater odds to be infected than Jade Ni breed. Grazing goats had 2 times more likely to be infected than non-grazing goats. Goats drinking common water had more likely to be infected than goats drinking well water. There was no association between mixed raising with other livestock and the presence of infection.

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Conflict of interest

The authors declare that they have no competing interests.

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