Subclinical Bovine Cryptosporidiosis and its Potential Risks in Cattle within Yangon Region

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DOI: 10.29322/IJSRP.12.08.2022.p12809
http://dx.doi.org/10.29322/IJSRP.12.08.2022.p12809

Paper Received Date: 12th July 2022
Paper Acceptance Date: 28th July 2022
Paper Publication Date: 6th August 2022

Abstract- A cross-sectional study was conducted to find out the prevalence and potential risk factors for shedding of *Cryptosporidium* spp. oocysts in cattle within Yangon Region. For this purpose, 350 fecal samples of cattle were collected from five quarters within Yangon region. The microscopic examination was performed with modified Ziehl-Neelsen (Z-N) staining. Pearson Chi-square test was used to determine potential risk factors. Among the fecal samples examined, 53.1% (186/350) were positive for *Cryptosporidium* spp. oocyst. Less than 6mth old calves had 2 times higher probability than the age group of 6mth-2yrs (CI: 1.04-3.7, p: 0.000, OR: 1.96) and had 13 times more likelihood than age group of >2yrs (CI: 1.02-4.1, p: 0.03, OR: 12.8). High risk of exposure to *Cryptosporidium* spp. infection was occurred in cattle of using river water supply (CI: 1.03-3.6, p: 0.05, OR: 2.97). Since *Cryptosporidium* spp. infection is zoonotic which can also cause major loss of productivity, routine fecal examination and control measures should be provided.

Index Terms- cattle, *Cryptosporidium* spp., microscopy, Ziehl-Neelsen

I. Introduction

Cryptosporidium spp., an intracellular coccidian parasite, become an interesting emerging infection for its opportunistic behavior in immunodeficiency patients (Petersen, 1992). *Cryptosporidium* spp. were recognized worldwide that can cause chronic and severe diarrhea (Fayer and Ungar, 1986). Regarding the impact on socioeconomic development, *Cryptosporidium* spp. has been included in the "Neglected Disease Initiative" of the World Health Organization (Geurden and O'Handley, 2011). Growth impairment, physical fitness and cognitive function in early childhood have been associated with *Cryptosporidium* spp. infections (Guerrant *et al*., 1999). Transmission mode could be either direct contact or consumption of contaminated food and water indirectly (Dixon, 2009). In Myanmar, 3.4% of cryptosporidiosis was occurred previously in infants between 2-11 months of age with acute diarrhea (Aye *et al.*, 1994).

On the aspect of conventional microscopic techniques, modified Ziehl-Neelsen (ZN) staining has been used to determine *Cryptosporidium* spp. oocyst in fecal samples even the sensitivity was low (Omoruyi et al., 2014). Apart from microscopic examination, sandwich antigen detection enzyme linked immunosorbent assay (sad-ELISA) and molecular base technology have been evaluated. However, their cost, technician involvement and time-consuming made conventional microscopic still preferrable one.

Cattle have been investigated as potential source for human cryptosporidiosis (Inpankaew *et al.*, 2010). Globally, *Cryptosporidium* spp. has been recognized as major causal organism of neonatal enteritis in calves (Mosier and Oberst, 2000). In Myanmar, 57.3% of calves within Mandalay Region has been tested as positive to *Cryptosporidium* spp. infection. In 2009, the overall prevalence of 56% has been reported in cattle from Yamethin and Pyawbwe Townships, Mandalay Region (data not published). Apart from central part of Myanmar, 100% in cattle, 52.5% in buffalo and 24.6% in mithun have been reported in Matupi Township, Chin State (data not published). However, the information on prevalence of *Cryptosporidium* spp. infection in cattle within Yangon Region has not been identified yet whereas Yangon is the second largest cattle industry in Myanmar (LBVD, 2019). Therefore, this study aimed to determine the prevalence of *Cryptosporidium* spp. infection in cattle within Yangon Region and to access the associated risk factors. This information may provide knowledge of infection within region and support for control measures.

II. Materials and Methods

2.1 Sample size calculation and sample collection

A cross sectional study was carried out within five villages in Yangon region (16.8409° N, 96.1735° E) in June – September 2017 (Figure. 1). The sample size was calculated using the formula described by Daniel, 1995 with estimated prevalence of 50%. The demographic data of sample animals was shown in (Table. 1). Amount of 25g fecal sample was collected per rectum directly. Separate disposable gloves and zip-locked plastic bags were used for sample collection. Then, the bags were labeled with cattle individual identity and were carried to Parasitology Laboratory of

Livestock Breeding and Veterinary Department, Mingaladon Township, Yangon using ice boxes. The samples were kept at 4°C refrigerator and examined within 12 hr after collection.

Figure 1. Geography map of study area; Yangon Region

Table 1. Demographic data of study animals

Demographic data	% (n)
Gender	
Male	57.1 (200/350)
Female	42.9 (150/350)
Age	
>6months	44.9(157/350)
6 mth – 2 yrs	29.7 (104/350)
>2yrs	25.4 (89/350)
Breed	
Local	53.7 (188/350)
Crossbred	46.3 (162/350)
Water source	
River water	57.7 (202/350)
Private wells	42.3 (148/350)

2.2 Identification of *Cryptosporidium* spp. oocysts by modified Z-N stain

Identification of *Cryptosporidium* spp. oocyst was conducted using modified Ziehl–Neelsen (Z-N) staining method (Clarke and McIntyre, 2001). Briefly, thin fecal smear was done, left air dried and fixed in the absolute 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 color disappeared. Thereafter, the slides were rinsed in tap water and counterstained with 0.25% malachite green for 30 seconds. Then, it was rinsed in tap water again, blotted or drained dry. Finally, the stained smear was examined for oocyst morphology using high power objective 40×. Regarding of oocyst scoring system of Dagnall Teaching Laboratory, Liverpool School of Tropical Medicine, oocyst density of each sample was determined as follows: rare (+) for less than 5 oocysts per slide; few to moderate (++) for 5-10 oocysts per field of view; and numerous (+++) for up to 11 oocysts per field of view. For each smear, 20 viewing fields covered at 1,000× magnification.

2.3 Statistical analysis

Data files of questionnaires and laboratory results were entered into Microsoft Excel Sheet. Pearson Chi-square test was used to identify hypothesized risk factors of *Cryptosporidium* spp. infected animal by using SPSS version 17 at 95% Confidence Interval (CI).

III. Results and Discussion

Out of 350 fecal samples, 186 samples (53.1%) showed *Cryptosporidium* spp. infection positive. The overall prevalence of *Cryptosporidium* spp. infection was found 53.1% in current study, which was similar to the previous findings of 57.3% in Mandalay city (Lay *et al*., 2008), 56% in Pyawbwe and Yamethin Townships (Bawm *et al*., 2014). Interestingly, all of the infected animals did not show any clinical symptoms.

In current study, prevalence of above 50% shown to be relatively higher than compared to neighboring countries, 0.6%, 12.5% and 11.9% in Thailand (Inpankaew et al., 2010, India (Baht et al., 2012) and China (Gong et al., 2017), respectively. Since molecular characterization of *Cryptosporidium* on species level in cattle haven't done yet in Myanmar and thus, the zoonotic potential of bovine cryptosporidiosis becomes critical for further detail.

Oocyst density of *Cryptosporidium* spp. in the study area was observed as 45.7% (160/350) for + and 7.4% (26/350) for + +. There was no samples showed + + +. In present study, most of all positive animals were asymptomatic and therefore, it could be noted that those animals were subclinically infected. As single oocyst is sufficient to produce infection and disease in susceptible hosts, the infected animal could serve as reservoir (Pereira *et al*., 2002).

According to statistical analysis, factors of age and water supply were associated with the presence of *Cryptosporidium* spp. infection (Table. 2). In detail, less than 6mth old age had the highest prevalence rate 78.3% (123/157) followed by 6mth – 2yrs of 35.6% (37/104) and >2ys of 29.2% (26/89). The calves less than 6mth had 2 times more likely to be infected than 6mth-2yrs and 13 times more likely to be infected than >2yrs. In calves, there was more chance on occurrence of oocyst because of viability of oocyst in soil have been reported up to 60 days (Lim *et al.*, 1999). Therefore, sanitation management of calves and their manure could be critical to prevent infection. Apart from that, high prevalence rate in calves could be due to their low immune response and thus susceptible to infection (Thompson et al., 2017). This information corroborates with previous reports of Quilez *et al.*, 1996, Lefay *et al.*, 2000 and Castro-Hermida *et al.*, 2002 in which the prevalence was significantly higher in newborn and suckling calves as oocyst shedding was negatively correlated with age Nasir *et al.* (2009).

Table 2. Association between *Cryptosporidium* spp. infection and potential risk factors

No.	Risk factors	Positive		p- value	OR (95%CI)
		Number of animals	Prevalence (%)		
1.	Gender				
	Male	84	42.0		
	Female	102	68.0	0.98	1.79 (0.98-3.2)
2.	Age				
	<6 mth	123	78.3		
	6 mth-2 yrs	37	35.6	0	1.96 (1.04-3.7)
	>2 yrs	26	29.2	0.03	12.8 (1.02-4.1)
3.	Breed				
	Indigenous	97	51.6		
	Crossbred	89	54.9	0.57	1.78 (0.12-0.63)
4.	Water source				
	River water	133	65.8		
	Private wells	53	35.8	0.05	2.97 (1.03-3.6)

On behalf of breed, there was no difference between indigenous and crossbred. Thus, breed factor had been shown without any association with the presence of infection as previously described (Ayele et al., 2018). Moreover, sample size of our study reflects gender equality thus, it couldn't favor to show significant difference between male and female.

Regarding to our findings, the farm with river water supply had more chances to get infection compared to private wells. Since the grazing pastures were supplied by river water, it could probably be infected to the farming site as different way of infection (Bawm et al., 2014). In current study, all of the sample animals were kept intensive farming and feed with cut and curry system. Apart from that, the source of water for both human and animal come from the river nearby Yangon. Therefore, it could be possible that water troughs might serve as reservoir because drinking of contaminated river water have been reported as waterborne route of *Cryptosporidium* spp. infection (Shirley et al., 2012). Sanitation practice and proper handling of cattle manure could be improved since excreted feces from infected animal found to be infected others (Vermeulen et al., 2019).

The present finding was the first investigation on the prevalence of *Cryptosporidium* spp. infection in cattle within Yangon region. Our study pointed out that calves were being at high risk of infection than older ones. As public concern of this parasite, awareness on prevalence and associated factors for shedding of *Cryptosporidium* spp. oocyst might be helpful in designing prevention strategies. To address their impact, molecular characterization should be performed in order to

identify species level. Moreover, assessment of potential risk relevant with human infection are also necessary to find out.

IV. Conclusion

According to present study, the overall prevalence of bovine *Cryptosporidium* spp. infection within Yangon region was 53.1% in which *Cryptosporidium* spp. infection was more well encountered in calves younger than 6 months. Out of positive cattle, 45.7% were shed *Cryptosporidium* spp. oocyst with rare rate (+) while 7.4% of cattle shed moderately (+ +). Contaminated river water supply could be investigated further for public health concerns as the cattle used river water supply found to be infected significantly. Although we haven't done for molecular characterization, this study could benefit in designing control measures.

Declaration of Competing Interests

The authors declare that there are no competing interests.

Acknowledgements

The authors would like to express their gratitude to Township Veterinary Officers and laboratory staffs from Livestock Breeding and Veterinary Department, Mingaladon Township, Yangon for their kind support during sample collection and identification.

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