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Epidemiological Study of Small Ruminant *Cryptosporidium* Infection in Ziway Dugda District of East Arsi Zone, Ethiopia

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Abstract

This study was undertaken to determine the prevalence and intensity of small ruminant *Cryptosporidium* infection and to investigate the role of potential risk factors associated with the occurrence of the disease in Ziway dugda district of east arsi zone, Ethiopia. Faecal samples were collected from 174 sheep and 210 goats under the age of one year. Samples were analysed using the sheather's sugar solution flotation technique and Modified Ziehl–Neelsen staining technique. 59 samples were found positive giving an overall prevalence of 15.4%. Significant difference (P<0.05,) was observed in the prevalence of small ruminant *Cryptosporidium* infection among poor, medium and good body condition animals (p-value=0.004), and in between diarrheic and non- diarrheic animals (p-value=0.002). However, all the risk factors considered in this study had no significant effect (p- value>0.05) on the prevalence of *Cryptosporidium* infection. Regarding the intensity of the infection, 31 samples (8.1%) were scored as "high," 17 (4.4%) were scored as "moderate," and 11 (2.9%) were scored as "low," while the remaining 325 samples (84.6%) were "negative". The intensity of *Cryptosporidium* infection is significantly higher in small ruminants having poor body condition (p-value=0.038) and diarrhea (p-value=0.025). This study demonstrated the importance of *Cryptosporidium* infection in small ruminants less than one year of age and having diarrhea and poor body condition in Ziway dugda district of east arsi zone, Ethiopia.

Keywords: *Cryptosporidium*; Small ruminants; Prevalence; Intensity; Ziwaydugda

Introduction

Parasitic infections pose a serious health threat and remain one of the major impediments to small ruminant production in many part of the world including Ethiopia [1]. *Cryptosporidium* infection is one of the most significant, zoonotic, parasitic diarrhea causing disease in many agro-ecological zones being a serious threat to the livestock economy worldwide [2,3]. It is recognized as a major constraint to livestock production throughout the tropics and elsewhere [4].

Cryptosporidium is monoxenous life cycle that causes diarrhea in immunocompromised individuals and neonates that result from parasite invasion and epithelial destruction with the result of mild to moderate villus atrophy and microvillii shortening and destruction [5]. *Cryptosporidium* oocysts are transmitted between hosts via the fecal-oral route; either directly from contact with faeces of infected animals or indirectly through environmental contamination or from ingestion of contaminated food or water and it takes less than 50 oocysts to infect a healthy animal [6]. Whereas age, body condition score, immune status, concurrent infections, management and hygienic conditions are the potential risk factors [7,8].

Currently, up to 14 species of *Cryptosporidium*, infecting mammals, fish, and birds, have been proposed but only two of these are of importance to agricultural animals. These include *C. paruum* which infects many different hosts including cattle, swine, horses, and small ruminants, and the calf genotype of *C. muris*, now called *C. andersoni*, which infects cattle [8].

A variety of methods is available for detection of *Cryptosporidium* species including microscopic, immunological and molecular techniques. Microscopic detection is based on finding the environmental and chemical resistant oocysts in fecal samples [9]. The demonstration of oocysts concentrated from fecal samples is by centrifugal flotation in high specific-gravity salt or sugar solutions. The modified Ziehl-Neelsen is a simple and rapid procedure well suited for large-scale routine diagnosis of *Cryptosporidia* [8].

Information about the prevalence and associated risk factors of small ruminant *Cryptosporidium* infection is an essential point to design and implement control strategies. Although considerable work has been done on small ruminant gastrointestinal parasitic infection in Ethiopia, specific studies that indicate the prevalence and distribution of *Cryptosporidium* infection are scant. Furthermore, in contrast to the vast studies on bovine cryptosporidiosis, the occurrence of the disease in small ruminants has received little attention. The parasite is however considered as one of the major enteric pathogens associated with neonatal diarrhea and mortality in sheep and goats [10,11]. Hence, this study was conducted to determine the prevalence and intensity of small ruminant *Cryptosporidium* infection and to investigate the role of potential risk factors associated with the occurrence of the disease in Ziway dugda district of east arsi zone, Ethiopia.

Materials and Methods

Study area

The study was conducted from November 2017 to April 2018 in Ziway dugda district, East Arsi zone of Oromia regional state, Ethiopia. The area is located 221 km south East of Addis Ababa, the capital city of the country and 46 km from Asella, the capital city of East Arsi zone. The district is in the great rift valley of Ethiopia. Ziway dugda district has an area of 1269.07 km^2 with 31.7% is arable or used for crop cultivation, 6% of pasture, 46.3% forest and the remaining 16%

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is swampy, mountainous or unusable. Topographically, the district is tropical in nature located between 8° 05'N-8°25' N latitude and 39°E-39°45' E longitudes at an altitude of 1600 to 1800 m above sea level with the minimum and maximum temperature 19°C and 32°C respectively. The district receives an average annual rainfall ranges between 650 to 800 mm, with bimodal rainfall March to April (short rainy season) and July to October (long rainy season). Estimated animal population in the area is about 124,680 cattle, 24,524 sheep, 40,286 goats, 17,851 equines and 60,345 chickens [12].

Study population and study protocol

A total of 384 small ruminants consisting of 156 males and 228 females were examined for *Cryptosporidium* infection, out of which 174 were sheep and 210 goats. Furthermore, 112 of the study animals were found diarrheic but the rest 272 were non-diarrheic. All of the animals in the study were local breeds kept under extensive management system and had not received any anticryptosporidial medication prior to sampling.

The study was conducted using clinical and laboratory examinations techniques. During the clinical examination, the species, sex, age and body conditions of the study animals were recorded. All clinical findings, particularly GIT syndromes, were recorded and fecal samples were collected from each animal for coprology. Laboratory examination was conducted by sheather's sugar solution flotation technique [13] and Modified Ziehl–Neelsen staining technique [14] for *Cryptosporidium*.

To ease statistical analysis, the animals were classified into three age groups: 0- 1 month (very young), 1 month- 6 months (young) and 6 months-1 year (young adult). The animals were also classified as poor, moderate and good based on the appearance of their body condition and manual palpation of the spines and transverse processes of lumbar vertebrae as described by Morgan et al. [15]. The age of sheep and goats were determined based on owners' response and using dentition [16].

Study design and sampling method

A cross-sectional study was carried out from November 2017 to April 2018 to determine the prevalence and to investigate potential risk factors of *Cryptosporidium* infection in sheep and goats under one year of age in Ziway dugda district, East Arsi zone of Oromia regional state. The study district was selected based on higher concentration of small ruminants and accessibility. A simple random sampling technique was employed for selection of the study animals. The desired sample size for the study was calculated using the formula given by Thrusfield [17] with 95% confidence interval and 5% absolute precision.

$$N = \frac{1.962 \times \text{Pexp}\left(1 - \text{Pexp}\right)}{D^2}$$

Where; N=sample size, Pexp=expect prevalence, D=absolute precision (5%).

A 50% expected prevalence was taken since there is no previous report on the prevalence of small ruminant *Cryptosporidium* infection in the study area. Accordingly, 384 animals were included for the study.

Sample collection and Sample processing

Faecal samples were collected directly from the rectum using plastic gloves and put into clean, dry, leak-proof, transparent plastic bottles. For animals in which rectal sampling was not possible, such as neonates and diarrheic, freshly voided faeces were collected by the use of wooden tongue depressors. The samples were labelled and transported to Asella animal disease survey, investigation and diagnostic laboratory where they were examined immediately for *cryptosporidium* oocyst. Fecal samples that were not observed on the same day were treated and stored in the refrigerator for subsequent examination the next day. Sampling was done according to Akinkuotu and Fagbemi [4].

The Sheather's floatation technique (SFN) as described by Trotz-Williams et al. [18] was used to detect the presence of *Cryptosporidium* oocyst. Fecal samples containing *Cryptosporidium* oocyst were then subjected to microscopic examination of smear using Modified Ziehl Nelson's acid fast staining technique (MZN) [19]. Oocysts which appeared bright red granules on a blue background were taken as positive. If no oocysts were detected, it was scored as negative [20].

The intensity of the infection was estimated semi quantitatively according to the average number of oocysts in 10 random fields. It was scored as light (<5 oocysts/10 fields), moderate (5–10 oocysts/10 fields), and high (>10 oocysts/10 fields). If no oocysts were detected, it was scored as negative [21].

Statistical analysis

The data collected was entered in Microsoft excel work sheet and analyzed using IBM SPSS 20.0 2011 software for Windows (IBM SPSS Corp., Armonk, NY, USA). Chi-square test was used to determine the relationships between studied risk factors and sample positivity. A P-value ≤ 0.05 was considered statistically significant.

Results

Of the total 384 fecal samples tested using sheather's sugar flotation technique, 37.8% (n=145) were found positive for *Cryptosporidium*. Of these, 15.4% (n=59) were confirmed to be positive for *Cryptosporidium* up on further testing by a modified Ziehl-Neelsen staining technique giving 22.4% overall false positives. Thus, the overall prevalence of *Cryptosporidium* infection in this study was 15.4% (n=59). Animals having a poor body condition (n=147) had higher prevalence of *Cryptosporidium* infection (p-value=0.004) than those having moderate (n=160) and good (n=77) body condition. In addition, higher prevalence of *Cryptosporidium* (p-value=0.002) was observed in animals with diarrhea (n=112) than the non-diarrheic ones (n=272). However, all the risk factors considered in this study had no significant effect (p- value>0.05) on the prevalence of *Cryptosporidium* infection.

Regarding the intensity of the infection, 31 samples (8.1%) were scored as "high", 17 (4.4%) were scored as "moderate", and 11 (2.9%) were scored as "low", while the remaining 325 samples (84.6%) were "negative". The intensity of *Cryptosporidium* infection is high (p-value=0.038) in small ruminants having poor body condition than others. Similarly, a higher intensity of *Cryptosporidium* (*p-value=0.025*) was recorded in diarrheic than non- diarrheic. The intensity of occurrence and prevalence of *Cryptosporidium*, and their association with different risk factors are summarized in Tables 1 and 2.

Discussion

In this study, out of the 384 fecal samples examined, 15.4% (n=59) were positive for *Cryptosporidium* oocysts, with 17.8% (n=31) and 13.3% (n=28) collected from sheep and goats, respectively. This finding was comparable to the previous observation of Mahdi and Ali [22] who reported 17.7% *Cryptosporidium* infection in small ruminants. In this study however, the prevalence of *Cryptosporidium* infection in sheep was higher than the findings by Mahfouz et al. [23], Maurya et al. [11] and Koinari et al. who had reported a prevalence of 2.5%, 1.8% and 2.2%, respectively. In the same way the prevalence of *Cryptosporidium* infection in goats in this study was higher than prevalence reported in

Variables	Category level	No. of examined Animals	No. of positives by *SFN	No. of positives by *MZN	*P-value*
Species	Ovine	174	71 (40.8%)	31 (17.8%)	0.225
	Caprine	210	74 (35.2%)	28 (13.3%)	
Sex	Male	156	66 (42.3%)	22 (14.1%)	0.571
	Female	228	79 (34.5%)	37 (16.2%)	
BCS	Poor	147	67 (45.6%)	34 (23.1%)	0.004
	Moderate	160	54 (33.3%)	18 (11.1%)	
	Good	77	24 (31.2%)	7 (9.1%)	
Fecal consistency	Diarrheic	112	71 (63.4%)	27 (24.1%)	0.002
	Non-diarrheic	272	74 (27.2%)	32 (11.8%)	
Age	0-1months	193	77 (39.9%)	33 (17.1%)	0.423
	1months-½ year	116	42 (36.2%)	18 (15.5%)	
	½year-1year	75	26 (34.7%)	8 (10.7%)	
At 95% Confidence Interval: *SFN= Sheather's Floatation Technique, *MZN = Modified Ziehl Nelson's Acid-Fast Staining Technique					

Table 1: Distribution of *Cryptosporidium* infection among species, sex, body condition score (BCS), fecal consistency and age.

Variables	Category level	No. of exami ned Animals	High intensity	Moderate intensity	Light intensity	*P-value*
Species	Ovine	174	18 (4.7%)	6 (1.6%)	7 (1.8%)	0.218
	Caprine	210	13 (3.4%)	11 (2.9%)	4 (1%)	
Sex	Male	156	13 (3.4%)	6 (1.6%)	3 (0.8%)	0.781
	Female	228	18 (4.7%)	11 (2.9%)	8 (2.1%)	
BCS	Poor	147	20 (5.2%)	9 (2.3%)	5 (1.3%)	0.038
	Moderate	160	7 (1.8%)	6 (1.6%)	5 (1.3%)	
	Good	77	4 (1%)	2 (0.5%)	1 (0.3%)	
Fecal consistency	Non-Diarrheic	112	14 (3.6%)	8 (2.1%)	5 (1.3%)	0.025
	Diarrheic	272	17 (4.4%)	9 (2.3%)	6 (1.6%)	
Age	0-1month	193	18 (4.7%)	10 (2.6%)	5 (1.3%)	0.887
	1month-½ year	116	9 (2.3%)	5 (1.3%)	4 (1%)	
	½ year-1 year	75	4 (1%)	2 (0.5%)	2 (0.5%)	
At 95% Confidence Interval						

Table 2: The intensity of *Cryptosporidium* infection and its association among species, sex, body condition score (BCS), fecal consistency and age.

goats by Mahfouz et al. [23] and by Koinari et al. who have reported 0% and 4.4%, respectively. In another study, the prevalence of *Cryptosporidium* infection reported in goat kid by Bejan et al. [24] and Misic et al. [25] were 24% and 31.8%, respectively. In one study, the reported prevalence rate of *Cryptosporidium* infections in lambs was 21.05% by Gokce et al. [26]. The study conducted by Dinka et al. [27] on Eimeria and *Cryptosporidium* infections in sheep and goats at Elfora export abattoir, Central Ethiopia exceptionally reported zero prevalence of *Cryptosporidium* infections. The differences in the prevalence of small ruminant *Cryptosporidium* infections in this and previous studies may be the result of differences in the levels of contamination of the environment with oocysts of the parasite or may be due to differences in the infectivity of different *Cryptosporidium* species populations. It is also possible that the quality of hygienic conditions of animal husbandry and grazing practices may have influenced the exposure of animals to *Cryptosporidium* infection. The variations could also be due to the disparity in the susceptibility of the target population related to age difference, health status and hygienic practices [28]. Furthermore, the diagnostic tests utilized could also be the cause of this variation [29].

In this study, the prevalence of *Cryptosporidium* infection was higher in sheep (17.8%) than in goats (13.3%), similar to the observation by Waruru et al. [30]. However, there was insignificant difference (P>0.05, p-value=0.225) in the prevalence of *Cryptosporidium* infection between the two species of the study animals. The higher prevalence of *Cryptosporidium* infection in sheep in this and previous studies could be due to the feeding habits of these animals. That is, Goats are usually browsers in nature and they tend to graze in very rare cases where they do not find shrubs and bushes; thereby reducing the risk of being infected with sporulated oocysts of *Cryptosporidium* species and other internal parasites.

The prevalence of *Cryptosporidium* infection in this study varied insignificantly (P>0.05, p-value=0.571) with the sex of the animals. This is in agreement with the findings of Nooredeen et al. [31]. However, higher prevalence was recorded in females (16.2%) than in males (14.1%). The reason for this might be the practice by farmers to retain more females than males for the advantage of breeding and milk production.

There is a statistically significant difference in the prevalence of *Cryptosporidium* infection (p value=0.002) between diarrheic (24.1%) and non-diarrheic (11.8%) animals. This agrees with Lise et al. [32] and Maurya et al. [11] who reported a higher prevalence of *Cryptosporidium* in diarrheic than non-diarrheic animals. Of the 59 (15.4%) *Cryptosporidium* positive fecal samples 31 (8.1%) had a high intensity of infection; the majority of which is associated with diarrhea (p-value=0.025). This is in agreement with Delafosse, [33] and Giadinis et al. [21] who reported a strong association between *Cryptosporidium* oocyst shedding and small ruminant diarrhea. This is attributable to the fact that *Cryptosporidium* acts in concert with other enteropathogens to produce intestinal damage and diarrhea [8].

The prevalence rate of small ruminant *Cryptosporidium* infection among the very young (01 month), young (1 month- 6 months) and young-adult (6 month-1 year) age groups was 17.1%, 15.5% and 10.7%, respectively. There is no significant difference (p-value=0.423) in the prevalence of small ruminant *Cryptosporidium* infection among the age categories, which is in agreement with the reports by Dagnachew et al. [34], Fruiza et al. [35] and Bhat et al. [36]. On the contrary, several works have indicated that *Cryptosporidium* infection is significantly associated with neonates than adult animals [29,34]. The insignificant variation in prevalence of *Cryptosporidium* infection among the age groups in this study could be due to the extensive management system, where lambs and goat kids, irrespective of their age, are raised together with their parents under the same field conditions in the study area.

In this study the prevalence of *Cryptosporidium* infection in small ruminant with poor, medium and good body condition was 23.1%, 11.1% and 9.1%, respectively. The prevalence of *Cryptosporidium* infection vary significantly (p-value=0.004) among the body condition categories which was higher in animals with poor body condition. This is in agreement with a previous work by David et al. [37] and Swai et al. [38]. High intensity of *Cryptosporidium* infection (p-value=0.038) was observed in small ruminants with poor body condition compared to moderate and good body conditioned animals. This can be related to lowered immunity of poor body conditioned animals which are more susceptible to clinical disease than immunocompetent animals.

Conclusion

Cryptosporidium is prevalent among small ruminants less than one years of age in the study district. The study clearly showed variations in *Cryptosporidium* prevalence and intensity among the risk factors identified for the individual animal. A higher proportion of infection and greatest oocyst excretion is detected in poor body conditioned diarrheic goats and sheep less than one month age. Conclusively, cryptosporidiosis is very common in diarrheic goat kids and lambs having poor body condition. This study emphasizes the isolation of diarrheic goat kids and lambs during the course of the diarrhea and other possible control strategies aimed at minimizing transmission between the sources of the organism i.e., diarrheic goat kids and lambs and other animals at risk.

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