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 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; Ziway dugda

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 microvilli shortening and destruction [5]. Cryptosporidium oocysts are transmitted between hosts via the fecal-oral route; either directly from contact with feces 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. parvum 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 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.

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 km² with 31.7% is arable or used for crop cultivation, 6% of pasture, 46.3% forest and the remaining 16%...
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.

\[
N = \frac{1.96^2 \times \text{Pexp} (1 - \text{Pexp})}{\text{D}^2}
\]

Where: \(N\) = sample size, \(\text{Pexp}\) = expect prevalence, \(\text{D}\) = 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 Akinikuotu 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 floatation 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* infection (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 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...
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 Masic 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 Ellora 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. Variations in the susceptibility of the target population related to age, 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 Noordeen 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 among species, sex, body condition score (BCS), fecal consistency and age.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Category level</th>
<th>No. of examined Animals</th>
<th>No. of positives by *SFN</th>
<th>No. of positives by *MZN</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Ovine</td>
<td>174</td>
<td>71 (40.8%)</td>
<td>31 (17.8%)</td>
<td>0.225</td>
</tr>
<tr>
<td></td>
<td>Caprine</td>
<td>210</td>
<td>74 (35.2%)</td>
<td>28 (13.3%)</td>
<td>0.571</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>156</td>
<td>66 (42.3%)</td>
<td>22 (14.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>228</td>
<td>79 (34.5%)</td>
<td>37 (16.2%)</td>
<td></td>
</tr>
<tr>
<td>BCS</td>
<td>Poor</td>
<td>147</td>
<td>67 (45.6%)</td>
<td>34 (23.1%)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>160</td>
<td>54 (33.3%)</td>
<td>18 (11.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>77</td>
<td>24 (31.2%)</td>
<td>7 (9.1%)</td>
<td></td>
</tr>
<tr>
<td>Fecal consistency</td>
<td>Diarrheic</td>
<td>112</td>
<td>71 (63.4%)</td>
<td>27 (24.1%)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Non-diarrheic</td>
<td>272</td>
<td>74 (27.2%)</td>
<td>32 (11.8%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0-1months</td>
<td>193</td>
<td>77 (39.9%)</td>
<td>33 (17.1%)</td>
<td>0.423</td>
</tr>
<tr>
<td></td>
<td>1months-½ year</td>
<td>116</td>
<td>42 (36.2%)</td>
<td>18 (15.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>½year-1year</td>
<td>75</td>
<td>26 (34.7%)</td>
<td>8 (10.7%)</td>
<td></td>
</tr>
</tbody>
</table>

At 95% Confidence Interval: *SFN = Sheather's Floatation Technique, *MZN = Modified Ziehl Nelson's Acid-Fast Staining Technique

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

At 95% Confidence Interval

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 year 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 condition diarrheic goats and lambs 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.

**Acknowledgements**

The authors would like to thank Wollo University, School of veterinary medicine for the financial support. We are also grateful to the staffs of Assela animal disease investigation and diagnostic laboratory for collaboration in the laboratory work.

**References**


