Cryptosporidiosis in Calves, Lambs and Goat Kids in Bishoftu, Oromia Regional State, Ethiopia

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Abstract: A cross sectional study was carried out from November 2014 to April 2015 to determine the prevalence of Cryptosporidiosis in calves, lambs and goat kids and to assess potential risk factors in Bishoftu, of Oromia regional state, Ethiopia. Hence, fecal samples from a total of 364 study animals (214 calves, 89 lambs and 61 kids) were examined with Sheather’s flotation technique and Modified Ziehl-Neelsen Staining. Accordingly, the overall prevalence was found to be 14%. The prevalence of 13.6% (29/214) in calves, 16.9% (15/89) in lambs and 11.5% (7/61), in kids were recorded. There was a significant difference (P < 0.05) in the prevalence of cryptosporidiosis between diarrheic and non-diarrheic animals. There was no statistically significant variation (P>0.05) observed in the prevalence of Cryptosporidium infection between the study sites, animal species and among age groups. In conclusion, this study demonstrated the importance of Cryptosporidium in young ruminants with a higher prevalence among diarrheic animals than non diarrheic ones.

Key words: Bishoftu • Calves • Cryptosporidiosis • Kids • Lambs • Prevalence • Risk Factors

INTRODUCTION

Ethiopia possesses the largest livestock population in Africa. Livestock is a significant contributor to economic and social development in Ethiopia at household and national level. Livestock accounts for 15-17% of total GDP and 35-49% of agricultural GDP. Livestock directly contributes to the livelihoods of more than 70% of Ethiopians [1].

The country faces a range of opportunities and constraints improving the productivities of its livestock population. The main constraints to increasing livestock productivity and output are the lack of adequate supplies of good quality livestock food, high incidence of disease and mortality rates and water shortage. Livestock disease is the major constraints of productivity causing economic losses to the peasant farmers and pastoralists in Ethiopia community to hundreds of millions of birr annually [2].

Infectious diarrhea (scours) of neonatal animal is a common disease. The infectious agent that causes diarrhea can be a virus (Bovine viral diarrhea virus, rotavirus, coronavirus), bacteria (E. coli, Salmonella, enterotoxaemia), protozoa (coccidia, cryptosporidiosis, Giardia) [3,4].

Cryptosporidiosis is an emerging protozoan disease, caused by Cryptosporidium species, that can cause gastrointestinal infection in a wide variety of mammals including human, cattle, sheep, goat, pig and horses worldwide [5]. The infection being encountered after ingestion of the microscopic infective oocysts. Oocysts are discharged in the feces of infected cattle and are of primary importance for the dispersal and survival of the parasites [6]. Calves are primarily infected via the fecal-oral route and it takes less than 50 oocysts to infect a healthy calf [7]. Infection can rapidly spread from calf to calf when animals are commonly housed and overcrowded or from cow to calf via the udders when they are contaminated with infected calf feces in the lying area of the dams. These oocysts are resistant to the environment and remain infective for months in cold water or dump, cool environment [5].

Clinical Cryptosporidiosis is frequently not diagnosed, yet it has been incriminated as an important cause of diarrhea in neonates. Clinically, the disease is characterized by anorexia and diarrhea, often intermittent, which may result in poor growth rate. The severity of clinical disease may be associated with the animals’ immune and nutritional status. It is also characterized by

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low morbidity which, however may become severe when associated with other pathogens [5], although calves 1-3 weeks old seem to be most susceptible, Cryptosporidium species has also been found in cattle over two years of age impairing rate of gain in feedlot cattle and milk production in dairy cattle [8]. Cryptosporidiosis occurs primarily in neonatal calves, but also in lambs and kids [9]. One major species, C. parvum, infects both farm animals and humans [10]. Cryptosporidiosis is a fairly prevalent disease in many countries and the disease is one of the most economically important diseases especially in calves and kids in Turkey [11]. Calves at one to 15 days are at the highest risk [12]. The higher percentage of oocysts excreted is observed in 7-day-old calves. Animals of all ages can be infected, but diarrhea occurs only in young animals [13]. In young calves there is a significant relationship between season and infection [12]. Cryptosporidium infections have now been reported in most domestic animals as well as in a large variety of wild and captive animals since its first identification by Tyzzer. Most infections have been described in mammals and are attributed to C. parvum [14]. Young animals appear to be more susceptible to infection and disease, while infections in adult animals are often asymptomatic or do not occur. Similarly to human cryptosporidiosis, the common symptom in animals is yellow watery diarrhea which leads to dehydration, weight loss, fever and inappetence. Most (immunocompetent) animals recover within 1–2 weeks of infection with supportive fluid therapy [14]. Cryptosporidiosis in ruminant species is typically symptomatic in the young. Among cattle, calves are susceptible to infection shortly after birth and remain so for several months [15]. Infection in dairy calves is most often detected (via fecal oocyst shedding) between 8 and 15 days of age, whereas infection in beef calves most often occurs between 1 and 2 months of age [16,17]. Infection in lambs and goat kids is more common in animals under 1 month old [18]. Infection can be spread animal-to-animal by the fecal-oral route, usually when animals are housed together in an overcrowded environment, but contamination of udders and water supplies by feces is another common source of transmission in livestock. Transmission of the bovine genotype of C. parvum from calves to humans is established [19, 20]. It is estimated that 50% or more of all dairy calves will shed detectable numbers of oocysts and that the parasite is present on more than 90% of all US dairy farms [21]. This high prevalence of infected calves incites caution in drinking unpasteurized milk. Milk can be contaminated through mechanisms of poor udder hygiene and recent outbreaks of human cryptosporidiosis associated with drinking unpasteurized milk have been reported [22, 23]. Furthermore, the large number of oocysts excreted during infection helps to ensure a high level of environmental contamination. Although not always confirmed, cattle facilities are frequently blamed when Cryptosporidium is found in surface waters. Thus, cattle living in close proximity to rivers should be considered potential causes of waterborne contamination, as surface run-off does transport Cryptosporidium oocysts in soils to water sources [24]. For calves, lambs and kids Cryptosporidium has become a concern not only because of the direct economic losses associated with the infection, but also from a public health perspective because of the potential for environmental contamination with Cryptosporidium oocysts. It is this zoonotic potential, the acquisition of C. parvum by humans from fecal contamination by animals, which may have the greatest impact on the dairy industry and food of animal origin. In 1981, cryptosporidiosis was diagnosed in veterinary students who cared for calves with cryptosporidiosis [25]. Recent molecular studies of cryptosporidiosis in cattle have shown that three species and one genotype of Cryptosporidium are responsible for most cattle infections (C. parvum, C. bovis and C. andersoni and Cryptosporidium deer-like genotype) [26]. C. parvum is known to infect humans worldwide and is recognized as the major zoonotic Cryptosporidium species, whereas C. andersoni has been reported in humans only once [27]. The most prevalent species were C. parvum in pre-weaned calves, Cryptosporidium bovis has been described recently. C. bovis oocysts are morphologically indistinguishable from C. parvum oocysts. Cryptosporidium bovis is a highly prevalent species that infects primarily post-weaned calves [28]. Among parasitic diseases, coccidiosis and cryptosporidiosis are important protozoan parasites responsible for low productivity and mortality in small ruminants. Cryptosporidiosis, caused by the genus Cryptosporidium, is an important protozoan disease of young animals and humans with a cosmopolitan distribution. Contrary to coccidiosis, the disease is not host-specific and affects several species of animals [29]. These parasites do not require an external development stage, but are immediately infective when passed in the feces as thick-walled oocysts. Within hosts
such as calves, lambs and kids Cryptosporidium have both sexual and asexual reproduction as well as a complete life cycle within the calves, lambs and kids through thin-walled oocysts. Through this mechanism, the population of the parasite in the individual host is magnified [30].

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 [8]. Oocysts may be demonstrated using Ziehl-Nielsen stained fecal smears in which the sporozoites appear as bright red granules. In Ethiopia, there is limited information on the status of Cryptosporidiosis. Therefore, it is with this view and understanding that the present study is initiated with the following specific objectives:

- To determine the prevalence of Cryptosporidiosis in calves, lambs and kids in Bishoftu
- To identify risk factors associated with the disease

**MATERIALS AND METHODS**

**Study Area:** The study was conducted in Bishoftu, east shoa zone of Oromia region from November, 2014 to May, 2015. Bishoftu is located 45 kms south east of Addis Ababa. The area is located at 9°N latitude and 40°E longitudes at an altitude of 1850 meters above sea level in central high land of Ethiopia. It has an annual rainfall of 866 mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures are 26°C and 14°C respectively, with mean relatively humidity of 61.3% [31].

**Study Population:** Only young animals of age group below 12 month calves, lambs and kids were considered for this particular study. The association of the disease occurrence was determined in relation with different age category (5 days to 6 months and >6 month-12 months), study sites, animal species (calves, lambs and kids) and fecal consistency (diarrheic and non diarrheic) of calves, lambs and kids as well.

**Study Design and Sample Size Determination:** A cross sectional study design was used to determine the prevalence of Cryptosporidium in calves lambs and kids. Systematic random sampling technique was applied to select each study animal.

The sample size was determined using the formula given by [32] with a 95% confidence interval since there was a study taken by [33] and his prevalence was 17.6% and at 5% absolute precision. Therefore, the total sample size is 223. To increase, the precision level these number was increased to 364 with the proportion of 214 (13.6%) calves, 89 (16.9%) lambs and 61 (11.5%) kids.

\[
n = 1.96^2 p\times q/d^2
\]

where: 
- \(n\) = sample size, 
- \(p\) = expected prevalence, 
- \(d\) = precision

**Sample Collection:** Fresh fecal samples were randomly collected directly from the rectums of study animals using disposable gloves, placed in universal bottle and transported in ice box to Veterinary parasitology laboratory, CVMA, for processing on the same day. The samples were placed in suitable leak-proof plastic containers, tightly closed, labeled and transported to the laboratory for examination. The samples were first screened by Sheather’s Floatation Technique [34] and those found positives were confirmed by Modified Ziehl-Neelsen Stain [35].

**Data Management and Analysis:** The data were entered to excel spread sheet program to create a data base which was transferred to SPSS 20.0 Version software program of the computer before analysis. All the parameters measured were analyzed by using SPSS 20.0 statistical software. Significant level was determined at 95% confidence level (P< 0.05).

**RESULTS**

Among 364 animals examined using Ziehl-Neelsen staining technique, the overall prevalence of Cryptosporidium in all species was found to be 14%. The prevalence in calves was 13.6% (29/214) while the proportion in lambs and kids were 16.9% (15/89) and 11.5% (7/61), respectively (Table 1). Significant difference (P < 0.05) was observed in the prevalence of Cryptosporidiosis between diarrhoeic and non-animals. However, there was no significant (P > 0.05) difference observed in the prevalence of cryptosporidiosis among animal species, study sites (farms) and between the age group of the study animals.

An examination of the consistency of the fecal samples showed that 26.9% (98/364) of samples were diarrheic. Of these, 41.8% (41/98) tested positive for Cryptosporidium oocysts. The remaining 73.1% (266/364)
Table 1: The prevalence of Cryptosporidiosis by animal species

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Number examined</th>
<th>Number positive</th>
<th>%</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat Kids</td>
<td>61</td>
<td>7</td>
<td>11.5</td>
<td>0.960</td>
<td>0.619</td>
</tr>
<tr>
<td>Lambs</td>
<td>89</td>
<td>15</td>
<td>16.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calves</td>
<td>214</td>
<td>29</td>
<td>13.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>364</td>
<td>51</td>
<td>14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: The prevalence rate of Cryptosporidiosis by farms

<table>
<thead>
<tr>
<th>Sites (farms)</th>
<th>Number examined</th>
<th>Number positive</th>
<th>%</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethiopian milk and meat institute dairy farm</td>
<td>24</td>
<td>4</td>
<td>16.7</td>
<td>1.140</td>
<td>0.980</td>
</tr>
<tr>
<td>Ethiopian Agriculture Research center dairy farm</td>
<td>28</td>
<td>3</td>
<td>10.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genesis dairy farm</td>
<td>29</td>
<td>3</td>
<td>10.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALFA fodder and dairy farm</td>
<td>72</td>
<td>9</td>
<td>12.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tseday small holder dairy farm</td>
<td>15</td>
<td>2</td>
<td>13.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fair field integrated small holder farm</td>
<td>47</td>
<td>7</td>
<td>14.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extensive farm</td>
<td>149</td>
<td>23</td>
<td>15.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>364</td>
<td>51</td>
<td>14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Prevalence of Cryptosporidiosis by age group and fecal consistency

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number examined</th>
<th>Number positive</th>
<th>%</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 days- 6 months</td>
<td>327</td>
<td>44</td>
<td>13.5</td>
<td>0.823</td>
<td>0.247</td>
</tr>
<tr>
<td>&gt;6 months- 12 months</td>
<td>37</td>
<td>7</td>
<td>18.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal consistency</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrheic</td>
<td>98</td>
<td>41</td>
<td>41.8</td>
<td>86.184</td>
<td>0.000</td>
</tr>
<tr>
<td>Non-diarrheic</td>
<td>266</td>
<td>10</td>
<td>3.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

were non diarrheic, with 3.8% (10/266) of these testing positive (Table 3). There was statically significant difference (P < 0.05) between the numbers of diarrheic samples with Cryptosporidium oocysts compared to the non diarrheic samples. The prevalence of Cryptosporidium significantly (p < 0.05) varied with fecal consistency of study animals being ten times higher likelihood of occurrence in diarrheic compared to its chance of occurrence in non diarrheic (OR = 76.488). The prevalence rate of Cryptosporidium according to age groups of 5 days- 6 month and >6 month- 12 month were 13.5% (44/327) and 18.9% (7/37) respectively. There was no significant (P > 0.05) difference among the age categories (Table 3).

DISCUSSION

Out of 364 fecal samples collected during this study, the overall prevalence of Cryptosporidium in all species was found to be 14%. This result was nearly in agreement with Shoukry et al. [36]. He reported an overall 20% prevalence of Cryptosporidiosis in cattle, sheep, goats and buffalos in Egypt. In Iraq, similar finding was reported by Al-Dabbagh et al. [37]. In Turkey, the prevalence rate of 21.05% in neonatal lambs was reported by Gokce et al. [38]. The prevalence in calves was (13.6%) while the prevalence in lambs and kids were (16.9%) and (11.5%) respectively. There was statically significant difference (P < 0.05) between diarrheic (41.8%) and non diarrheic animals (3.8%). This was in agreement with the results of previous studies showing that calves infected by enteric pathogens can be subclinically infected [39].

In Europe, Cryptosporidium oocysts were detected in 42.1% of the examined lambs and 31.8% goat kids in Serbia [40]. The study conducted by Dinka Ayana et al. [41] on Eimeria and Cryptosporidium infections in sheep and goats at ELFORA export abattoir, Central Ethiopia exceptionally reported zero prevalence of Cryptosporidium. In another study, conducted in Romania, the presence of Cryptosporidiosis was observed in 24% of the goat kids [42]. The differences in the prevalence of Cryptosporidium infections in lambs and goat kids raised in different geographical regions 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 spp. 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. This was also due to differences in management of the animals and in origin of animals because animals for slaughter were
usually transported from different areas, intermittent excretion of Cryptosporidium oocysts, the difficulty in detecting oocysts and the type of study design selected [33].

Higher stocking rate enhances the infection since infected calves produce large numbers of oocysts into confined calf house ensuring a high environmental contamination [43]. In contrast, in the extensively reared and traditional husbandry system, calves remain in large outdoor paddocks or with dam on pasture, where oocysts are dispersed on a large surface and are exposed to direct sunlight, which reduces the oocysts viability, resulting in a reduced infection pressure [44]. The infection in sheep and goats is also common and severity of clinical symptoms varies, the infection often causes death of lambs and kids [45,46] (also mentioned that in the extensively reared and traditional husbandry system, animals remain at large outdoor paddocks or with dam on pasture, where oocysts are dispersed on a large surface and are exposed to direct sunlight, which reduces the oocysts viability, resulting in a reduced infection pressure [47].

The 13.6% prevalence of Cryptosporidium in calves in the current study was compared with previous works. Higher prevalence of 17.6% in central Ethiopia [33], 19.2% in Zambia [43], 17.9% in France [44], 35.5% in USA, 33.5% in Vietnam, 27.9% in UK, 28.5% in Sirlanka, 47.9% in Spain and [7], 70% in the US. However, it was higher than the figure reported by Fayer et al. [48] (11.9%) in USA. These differences in the prevalence among countries may be attributed to the difference in the stocking rate and husbandry system of livestock production system of the countries. Besides, these variations could also be due to the difference in the susceptibility of the target population that related to age difference, diarrheic status and hygienic practice. Furthermore, the discrepancy between the sensitivity of the diagnostic tests utilized might also be the cause of this variation [43].

It was also agreed by Xiao et al. [49] and Nguyen et al. [50] that although Cryptosporidium was observed among all age groups, the prevalence of the disease in calves less than 6 months is significantly higher than older cattle. Additionally, some of the synergic infection of enteropathogens such as Rota virus, Corona virus, Salmonella and E. coli can result in the immunocompromised condition and the newborn animal will be more susceptible to the Cryptosporidium infection [44].

CONCLUSION

Cryptosporidiosis is an emerging protozoan disease, caused by Cryptosporidium species that can cause gastrointestinal infection in a wide variety of mammals including human, cattle, sheep, goat, pig and horses worldwide. In this study four major factors were found to be studied with the risk of infection with Cryptosporidium. These include farm types, animal species, age category and fecal consistency of the animals sampled. Our study was identified (isolated) in calves, lambs and kids in the study area. The rate of isolation of Cryptosporidium statistically significance (P<0.05) between diarrheic and non diarrheic calves, lambs and kids in the study, but there was no significance difference in the prevalence rate of Cryptosporidium between the study farms, animal species, (calves, lambs and kids) and between different age groups (5 days-6 months and >6 months-12 months) of the study period.

Based on the above findings, the following recommendations were forwarded:

- Creation of public awareness on the possible existence of cryptosporidiosis is essential
- Further study using molecular technique to identify the species of the parasite

REFERENCES


