Calf Coccidiosis in Local Dairy Farming System in and Around Haromaya District, Oromiya Region State, Ethiopia

Izack Mohamed, Yimer Muktar and Solomon Shiferaw

Abstract: A cross-sectional study was conducted from November 2016 to April 2017 in and around Haramaya district including Haramaya University dairy farm to determine the prevalence of coccidiosis in calves and associated risk factors. Fecal sample were collected from a total of 384 calves with the age of birth to 12 months old by random selection. After collection, the samples were chained with cold and transported to laboratory and examined for the presence of Eimeria oocysts by floatation techniques. For positive sample, a solution of 2.5% potassium dichromate (K₂Cr₂O₇) was added to the feces containing the oocysts for sporulation and identification of the species. Out of 384 calves, 122 (31.8%) were found to be positive for Eimeria species. There was a statistically significant difference (P<0.05) in prevalence of coccidiosis among the various age categories, management system and hygiene status of calves. However, the sex, body condition of the animals and breed was not significantly associated (P>0.05) with the infection by coccidiosis. A total of 6 species were identified namely Eimeria bovis (22.7%), Eimeria zuernii (17.2%), Eimeria auburnensis (11.7%), Eimeria Canadensis (7%), Eimeria ellipsoidalis (5.5%) and Eimeria cylindrical (3.4%), in order of their relative prevalence. Percentage of single and multiple-species infection of Eimeria species has been investigated on infected calves. Out of the 122 calves positive for Eimeria species, 22.9% were infected with single species, while 55.7%, 14.8% and 6.6% were found to be infected with two, three and four species respectively. Generally 77.1% were infected with multiple Eimeria species, which were found in this study. The multiple-species infections per positive sample ranged from two to four species.

Key words: Calves · Eimeria · Haramaya district · Prevalence

INTRODUCTION

Livestock systems in developing countries are characterized by rapid change, driven by factors such as population growth, increases in the demand for livestock products as incomes rise and urbanization. Livestock currently contribute about 30 percent of agricultural gross domestic product in developing countries [1] and is becoming the fastest growing sub-sector of agriculture. Africa hosts 205 and 174 million sheep and goats representing 17% and 13% of the world total small ruminant population, respectively. The population of small ruminants in sub-Saharan Africa is estimated to be 274 million. The population of livestock found in Ethiopia is estimated to be 53.4 million cattle, 25.5 million sheep and 22.78 million goats [2].

Bovine coccidiosis has a worldwide distribution [3]. More than 13 species of Eimeria and one species of Isospora have been described to infect cattle and causes of coccidiosis. Of the 13 species recorded, two of the principal pathogens are E. zuernii and E. bovis [4]. Coccidiosis spreads from one animal to another by contact with infected feces and is one of the most alarming problems for calf rearing industry. The development of clinical coccidiosis in cattle mainly depends on factors like species of Eimeria, age of infected animal, number of oocysts ingested, presence of concurrent infections and type of production system and management practices [5]. In associations with other enteric pathogens, coccidian has been indicated as an important cause of diarrhea in calves [6].
Signs of the disease include anorexia, loss of weight and hemorrhagic and mucoid diarrhea. In severe cases, feces are liquid, bloody and may contain strands of intestinal mucosa. Animals may become emaciated, dehydrated, weak and listless. Rectal prolapse may result from straining without defecation. The clinical course of coccidiosis ranges from 4 to 14 days and the mortality rate may be as high as 24% in severe outbreaks. Death is primarily a result of diarrhea, which causes a loss of electrolytes and dehydration; however, hemorrhaging or secondary complications such as opportunistic infections may contribute to mortality. Animals that recover from severe infections may suffer permanent production losses [7].

The prevalence, species composition and importance of bovine coccidiosis have been documented in various countries of the world [8] reported 82.28% infection rate in the coastal plain area of Georgia (USA) [9] reported 87.8% infection rate in a sub humid tropical climate [10] reported 73.2% infection rate in Kashmir valley. In Ethiopia, [11], who reported outbreak of coccidiosis due to E. zurnii and an overall prevalence of 24.9% in a 5-year retrospective laboratory examination in cattle in a study conducted in the Abay Tana settlement dairy farm in BahirDar [12] reported an overall prevalence of 68.1% in cattle in Addis Ababa and Debre Zeit area, [13] reported an overall prevalence of 22.7% in calf in selected dairy farms of Dire Dawa, [14] reported an overall Prevalence of 31.9% in bovine in Kombolcha district of South Wollo, [15] reported prevalence of 51.42% Prevalence of calves coccidiosis in Jimma town dairy farms and [16] who reported 62.5% of this parasite and Associated Risk Factors of Calf Coccidiosis in and around Asela Town.

Although considerable work has been done on bovine coccidiosis in many countries of the world and few part of Ethiopia, still there is lack of information on the occurrence and losses associated with bovine coccidiosis. And there was no previous study carried out on prevalence and intensity of calf coccidiosis in the present study area, where mixed crop-livestock production system is the main form of agriculture. On the other hand knowing the current situation of coccidiosis in the area could be the basis for all possible actions including its control and prevention. Therefore, taking into account the significance of the parasite as one of the most important causes of economic losses, the objective of this study were:

- To determine the prevalence and associated risk factors with Eimeria infections of calves less than 1 year in study area.
- To identify species of Eimeria and to obtain baseline data so as to design effective control measures.

### MATERIALS AND METHODS

#### Description of the Study Area:

The study was conducted in Haramaya town, Haramaya University dairy farm and four kebele of Haramaya district of Eastern Hararghe, Oromia region. The district is located 508 km East of Addis Ababa. Topographically, it is situated at an altitude of 1600 to 2100m above sea level at 9°26’N latitude and 42°3’E longitudes with the mean annual temperature and relative humidity of 18°C and 68%, respectively. Small holder mixed farming system is the dominant mode of production of the farmers in the area. The district has about 76, 336 cattle, 65, 083 sheep and 84, 916 goats, 22, 355 donkeys, 356 camels and 89, 800 chickens. The area receives an average annual rain fall of approximately 900 mm, with a bimodal distribution pattern [17].

#### Study Animals:

The study animals includes; male and female calves of local and cross breeds kept under extensive and intensive management system, with less than one year of age belonging to each purposely selected from Haramaya town, Haramaya university dairy farm and four kebele of in and around Haramaya district. The age of calves was determined according to Pace and Wakeman [18], as well as by collecting information from dairy farm or animal owners and then conveniently categorized in to two groups: Birth up to 6 months and 7 up to 12 months respectively.

#### Study Design:

A cross-sectional study was conducted from November 2016 to April 2017 to determine the prevalence and to assess associated risk factors of Coccidiosis in and around Haramaya district.

#### Sample Size Determination:

The sample size required for this study was calculated based on sample size determination method for simple random sampling of infinite population based on [19] as follows:

\[
 n = \frac{(1.96)^2 p_{exp} (1-p_{exp})}{d^2}
\]

where,\( n \) = required sample size, \( p_{exp} \) = expected prevalence, \( d \) = desired absolute precision.

Since no previous study was undertaken in the study area, the expected prevalence was considered to be 50%. Accordingly, with 5% absolute precision at 95% confidence level, the number of calves required to determine the prevalence was found to be 384.
Study Methodology

Sampling Method or Sample Collection: Before sample collection Age, sex, breed, hygienic status, management system and body condition of the calves less than one year’s registered. Then after, a fresh fecal sample of about 20gm was collected from the rectum of each calf using sterile disposable plastic gloves. The sample was placed in a labeled clean glass bottle container and transported under cold chain to the parasitology laboratory on the same day and was kept at 4°C in a refrigerator until processing within 48 hours of arrival.

Assessment of hygienic status of calves: the hygienic status of calf pens and the calves themselves were assessed based on housing system (ventilation, draughts, group pens, heavy stocking), sanitation of bedding (soiled bedding) and body parts of the calves [20] and was conveniently categorized as poor and good.

Laboratory Investigation: Qualitative fecal examination was conducted using flotation technique for the detection of the oocysts of Eimeria using concentrated sucrose solution (Sheather’s sucrose solution) with specific gravity of 1.27 according to Hendrix [21] as follows: Approximately 2- 3g of feces was mixed with enough water in a plastic cup using tongue depressor to make a semisolid suspension. The mixture (feces and water) was strained through a tea strainer over a second plastic cup pressing out the liquid with tongue depressor. The contents of the second plastic cup was transferred in to a 15-ml centrifuge tube which then placed in to the centrifuge and spun for 3 minutes at 2000 revolutions per minute ( rpm), processing 6 samples at a time. Then the supernatant, which contains fats and dissolved pigments, was decanted and the sediment was re suspended using a stirring action with a wooden applicator stick after adding the flotation solution to ½ incur from the top of the tubes. The test tubes were inverted six times after inserting a rubber stopper to mix the solution thoroughly with the sediment. The tubes were then filled with flotation solution until a reverse meniscus was present; cover slips were added and centrifuged in variable- angled ( not a fixed – angled) centrifuge for 5 minutes. After centrifugation, each glass cover slip was briskly lifted up and placed on a clean glass slide, not allowing formation of air bubbles. The entire area under each cover slip was examined under a binocular microscope at 40×magnification.

Sporulation of Eimeria Oocysts: A solution of 2.5% potassium dichromate was added to each fecal sample, which contained most of the Eimeria oocyst in a beaker, mixed thoroughly with a wooden applicator and poured into a Petri dish. Each Petri dish was left on the bench in the laboratory to allow sporulation. Thereafter, every 24 h, the culture of oocysts was mixed thoroughly and with the aid of medicine dropper, a drop of the culture was placed on a glass slide, covered with a glass cover slip and examined under the microscope to determine when sporulation occurred. When sporulation of oocysts was completed after 14 days, the Petri dish containing oocysts was covered up and stored in a refrigerator at 5°C until needed. Identification of coccidian species will be based on the morphological features of the sporulated oocysts (size, shape, color and texture of oocyst wall, presence or absence of micropyile, polar cap) and time of sporulation with the aid of taxonomic keys [22, 23, 24, 25, 26].

Data Analysis: Data collected from study sites were entered and stored in a Microsoft excel spread sheet program and coded for analysis. Statistical analysis was done on Statistical Package for Social sciences (SPSS) version 20 statistical software. Descriptive statistics was computed. The prevalence was calculated for all data as the number of infected individuals divided by the number of sampled individual and multiplied by 100. Categorical data were analyzed first with the chi square (χ²) test for independence as a screening process was used to evaluate the association between the prevalence of coccidiosis and different risk factors. P- Value less than 0.05 (at 5% level of significance) were considered significant in all analysis.

RESULTS

Out of 384 fecal samples examined, 122 were positive for Eimeria oocysts and hence the overall prevalence was found to be 31.8%. Out of the six Haramaya district’s surveyed for coccidiosis, virtually all had one or more calves shedding Eimeria oocysts. Analysis of the potential risk factor for the occurrence of coccidia has revealed that there were a significant association of coccidia (P<0.05) with age, management and hygiene status of the calves. However, there is no significant association of coccidia (P>0.05) with sex, breed and body condition of calves during which sample was collected. (Table 1).

With regard to identification of Eimeria species, a total of six species were identified. The most prevalent species investigated during the survey Eimeria bovis (22.7%) and E. Zurnii (17.2%) followed by E. aubernensis (11.7%), Eimeria Canadensis (7%), Eimeria ellipsoidalis (5.5%) and E. cylindrica (3.4%) (Table 2).
Table 1: The status of *Eimeria* infection according to studied factors

<table>
<thead>
<tr>
<th>Studied Factors</th>
<th>Number Examined</th>
<th>Positive No. (%)</th>
<th>DF</th>
<th>( \chi^2 )</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>159</td>
<td>50 (31.4)</td>
<td>1</td>
<td>0.013</td>
<td>0.909</td>
</tr>
<tr>
<td>Male</td>
<td>225</td>
<td>72 (32)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 6</td>
<td>188</td>
<td>43 (22.9)</td>
<td>1</td>
<td>13.454</td>
<td>0.000</td>
</tr>
<tr>
<td>7-12</td>
<td>196</td>
<td>79 (40.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td>216</td>
<td>64 (29.6)</td>
<td>1</td>
<td>1.044</td>
<td>0.307</td>
</tr>
<tr>
<td>Cross</td>
<td>168</td>
<td>58 (34.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Management</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extensive</td>
<td>264</td>
<td>70 (26.5)</td>
<td>1</td>
<td>10.765</td>
<td>0.001</td>
</tr>
<tr>
<td>Intensive</td>
<td>120</td>
<td>52 (43.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hygiene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>175</td>
<td>68 (38.9)</td>
<td>1</td>
<td>7.448</td>
<td>0.006</td>
</tr>
<tr>
<td>Good</td>
<td>209</td>
<td>54 (25.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body cond.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>199</td>
<td>60 (30.2)</td>
<td>1</td>
<td>0.500</td>
<td>0.479</td>
</tr>
<tr>
<td>Good</td>
<td>185</td>
<td>62 (33.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>384</td>
<td>122 (31.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: *Eimeria* species identified and their respective prevalence in descending order

<table>
<thead>
<tr>
<th>Types of <em>Eimeria</em></th>
<th>Total animals examined</th>
<th>Positive No.</th>
<th>Prevalence(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. bovis</td>
<td>384</td>
<td>87</td>
<td>22.7</td>
</tr>
<tr>
<td>E. zuerni</td>
<td>384</td>
<td>66</td>
<td>17.2</td>
</tr>
<tr>
<td>E. aubernensis</td>
<td>384</td>
<td>45</td>
<td>11.7</td>
</tr>
<tr>
<td>E. canadensis</td>
<td>384</td>
<td>27</td>
<td>7.0</td>
</tr>
<tr>
<td>E. ellipsoidalis</td>
<td>384</td>
<td>21</td>
<td>5.5</td>
</tr>
<tr>
<td>E. cylindrica</td>
<td>384</td>
<td>13</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Table 3: Percentage of single and multiple-species infections of *Eimeria* species in infected calves

<table>
<thead>
<tr>
<th>No.of <em>Eimeria</em> Species</th>
<th>No. of calves consist d/t oocysts</th>
<th>Relative Occurrences (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>28</td>
<td>22.9</td>
</tr>
<tr>
<td>Two</td>
<td>68</td>
<td>55.7</td>
</tr>
<tr>
<td>Three</td>
<td>18</td>
<td>14.8</td>
</tr>
<tr>
<td>Four</td>
<td>8</td>
<td>6.6</td>
</tr>
<tr>
<td>Total</td>
<td>122</td>
<td>100</td>
</tr>
</tbody>
</table>

Percentage of single and multiple-species infections of *Eimeria* species has been investigated on infected calves. Out of the 122 calves’ positive for *Eimeria* species, 22.9% were infected with single species, while 55.7%, 14.8% and 6.6% were found to be infected with two, three and four species respectively. Generally 77.1% were infected with multiple *Eimeria* species, which were found in this study. The multiple-species infections per positive sample ranged from two to four species as shown in Table 3.

**DISCUSSION**

Coccidiosis is a common problem in cattle worldwide. It is an important and common disease in cattle; which is responsible for economic losses in worldwide [27]. This study indicate that, out of 384 fecal samples examined, 122 were positive for *Eimeria* oocysts and hence the overall prevalence was found to be (31.8%) and out of six Haramaya district’s surveyed for coccidiosis all (99%) had one or more calves shedding *Eimeria* oocysts. The overall Prevalence of (31.8%) obtained from this study was found to be higher than the previous reported prevalence; 20% by Kebadu [28] in the Debre Zeit, 22.7% by Bekele *et al.* [13], in Dire dawa. 24.2% Nagwa *et al.* [29] in Egypt. 24.9% The result of the present study is comparable and the same line with the result of Alemayehu *et al.* [14], 31.9% in Komboleha.

However, the result of the present study lower than reports of Abebe *et al.* [12], which was 68.1% in Central Ethiopia, in Asella 62.5% by Asfaw *et al.* [16], in Jimma 51.42% by Yadessa *et al.* [15] and 67.4% in a neighboring, Kenya by Munyua and Ngotho [30], 68% in Turkey by Arslan and Tuzer [31], 64.2% in Canada by Kennedy and Kraika [32], 86.3% in USA by Ernst *et al.* [8] and 87.8% in Mexico by Rodriguez-Vivas *et al.*[33]. The prevalence of coccidiosis recorded in this study was lower than some reports and also higher few reports which were conducted in different country. This variation is most likely attributed to the differences in agro ecology and husbandry practices of the study animals in different agro ecologies [34].
With regard to identification of *Eimeria* species, a total of 6 species were identified based up on the characteristics of the oocysts of cattle as described by Berto et al. [23] and Berto et al. [26]. The result of most pathogenic *Eimeria* species obtained in this study *E. bovis* (22.7%) and *E. zuernii* (17.2%) closely related with the report of Heidari et al. [35]. *E. bovis* (23.7%), followed by *E. zuernii* (19.2%). In this study, two pathogenic and most prevalent of *Eimeria* spp. including *E. bovis* (22.7%) and *E. zuernii* (17.2%) and nonpathogenic species including *E. auburnensis* (11.7%), *E. Canadensis* (7%), *E. ellipsoidalis* (5.5%) and *E. cylinderica* (3.4%) were less prevalent in study animals. This is in agreement with the finding reported by other researchers Ernst et al. [4]; Kasiman and Al-Shawa [36]; Ciceck et al. [37]; Farkas et al. [38]; Almeida et al. [39].

Percentage of single and multiple-species infections of *Eimeria* species has been investigated on infected calves. Out of 122 calves positive for *Eimeria* species, 22.9% were infected with single species, while 55.7%, 14.8% and 6.6% were found to be infected with two, three and four species respectively. Generally 77.1% were infected with multiple *Eimeria* species. In this study demonstrated that mixed infections with two to four *Eimeria* species were more commonly observed. This finding is consistent with the findings of other researchers [40, 12]. The positive pair-wise interactions that found in this study may be result from synergistic effect of concurrent parasite infection. This study confirmed that the pathogenic coccidian, *E. bovis* and *E. zuernii* are the highest prevalent species in study area.

Analysis of risk factor in the association of disease occurrence has revealed that there was a strongly significant association (P<0.05) between the age of the calves with the risk of infection in which the prevalence of coccidia appeared to follow an age pattern. Higher infection rate was observed in calves 7 to 12 months of age than calves of birth to 6 months of age due to the fact that there was good nursing of the colostrum feeding for younger calves. During investigation, most calves older than 6 months were housed in overcrowded condition, less care were given and have easy contact with adult animals. This has given more chance for the animals to lick each other and ingest large number of oocysts, which is in agreement with previous reports [12, 33, 34, 41]. Coccidiosis occurs most commonly in young animals with a seasonal incidence when young calves are brought together for weaning or moved into feedlots or fed in small areas for the winter months. The prevalence of infection and the incidence of clinical disease are also age related [34].

The overall infection prevalence of *Eimeria* spp. of calves in female (31.4%) was slightly lower than that in male (32%), So that, the sex of the animals was not significantly associated (P > 0.05) with the infection. In this case, the results agree with Bekele [13], Yadessa et al. [15], Alemayehu et al. [14] and Asfaw et al. [16] but the prevalence obtained on male (32%) was bit higher than female (31.4%) which contradict with the work of above explained authors. The absence of a significant association between infection and animal sex might suggest that both male and female animals have an almost equal likelihood of being infected with coccidian. But lower prevalence on female than male in this study might be due to sample size, which number of examined male is higher than female.

The stronger association (P<0.05) of *Eimeria* infection in relation to the hygienic status (Good 25.6% and Poor 38.9%) of calves has been demonstrated in this study. Consequently, calves belonging to poor hygiene showed significantly higher prevalence than calves belonging to good hygiene. This result agrees with the report of Bekele et al. [13] and Asfaw et al. [16]. This could imply that poor sanitation in the calving and calf housing areas as well as poor management of housing favors infection with coccidiosis. Obviously, poor ventilation, droughts, poor calf nutrition, group pens, heavy stocking, cows present with calves, soiled bedding were regarded as risk factors for coccidiosis [42].

During investigation of risk factor, breed (Local 29.6%, Cross 34.5) in the association of disease occurrence has revealed that there was no statistically significant association (P>0.05) between breed and coccidia infection. These indicate that body condition does not have influence on the occurrence of coccidia infection. These results agree with the report of Abebe et al. [12] and Alemayehu et al. [14]. This is due to either equal chance of accessing the oocysts or no difference on protective immunity for the disease.

During investigation there was no a statistical significant association between body condition of the animals and coccidian infection (P>0.05). This result agrees with report of Fraser, [43] and Alemayehu et al. [14]. These indicate that body condition does not have influence on the occurrence of coccidia infection. This is due to either the level of infection, sampled size or most of the affected animals harbor the disease without showing clinical signs.

The influence of management system on prevalence of coccidia (Extensive 26.5% and Intensive 43.3%) has revealed that there was statistically significant association between them (P<0.05). This result also disagrees with the
previous report by Alemayehu et al. [14] indicating that there was no statistical significant association between the occurrence of coccidial infection and management system. This might be attributed to the fact that hygienic system of the barn, nutritional status and contamination of the feed or overcrowding of the animal was different in all management systems. Moreover, management factors may also be related to greater susceptibility of cattle to coccidial infection. Calves that are reared under artificial conditions are exposed to greater numbers of risk factors for eimeriosis, such as: early weaning, failure to ingest colostrum and difficulty in adapting to artificial high-density diets. Pasture with high concentrations of animals also present greater quantities of feces deposited and consequently, greater contamination of the ground with parasite eggs and oocytes, which constitutes a risk for susceptible calves [44].

CONCLUSION AND RECOMMENDATION

This study has revealed that coccidiosis is prevalent parasite of calves in and around Haramaya district. Six Eimeria spp, namely E. bovis, E. zuernii, E. auburnensis, E. Canadensis, E. ellipsoidalis and and E. cylinderica were identified in all Eimeria positive fecal samples. The high prevalence of Eimeria spp. was considered as one of the important infection in calves in the study area. The prevalence of coccidia has no significant association with sex, breed and body condition. However, the disease has a significant association (P<0.05) with age, hygiene and management being significantly higher in younger animal and with poor hygiene. Results from this study indicate the Eimeria infection has a great significance for the livestock producer and need a serious control and preventive issue. Therefore, further epidemiological investigation on coccidian species should be conducted in the study area.

Based on above conclusion, the following recommendation forwarded.

- Isolation and treatment of sick animals to prevent further disease and premise contamination.
- Hygiene of animal and premises should be regularly practiced.
- Special care should be given to young calves.
- Hygienic status of extensive as well as intensive management practice should be improved.
- Any risk factor that predispose calves to infection should get necessary action to prevent and control

REFERENCES


