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Prevalence and Risk Factors of Cryptosporidiosis in Dairy Calves in Asella Town, South Eastern, Ethiopia

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Abstract: A cross-sectional study was conducted from November 2014 to February 2015 in Asella, South eastern, Ethiopia, to study the prevalence of cryptosporidiosis in dairy calves and to assess the associated risk factors. Faecal samples were collected from 384 randomly selected calves (127 extensive, 164 semi-intensive and 93 intensive management systems) and examined by using modified acid-fast staining techniques. The overall prevalence of cryptosporidium positive calves was 24.0%. Prevalence of cryptosporidium infection in poor, medium and good body condition were 30.4%, 20.2% and 8.0%, respectively with statistically significant variation (x2= 8.849, P = 0.012). Breed of calves was also found to have a statistically high significant association with the prevalence of cryptosporidium infection (P < 0.05), the prevalence being high in exotic breed which is 38.3% (18/47), while 24.9% (67/269) and 10.3% (7/68) within, cross and local breed respectively (x2 = 12.407, P = 0.002). The occurrence of the disease was also assessed among management systems of animals by taking into consideration different management systems such as extensive, intensive and semi-intensive management system. The prevalence was (25.2%) in extensive, 30.1% in intensive and 19.5% in semi-intensive management system with absence of significant variation (P = 0.148). The sex and age of the calves was also found to have no significant association with prevalence of cryptosporidiosis (P > 0.05). A prevalence of 24.8%, 23.8% and 22.9% were observed in 4, 5-8 and 9-12 months age groups respectively. While the highest and lowest prevalence with in a sex was 26.7% and 20.8% in female and male respectively with p = 0.176. Therefore, even though there was a high disease occurrence among age groups in calves under 4 months than 5-8 and 9-12 month age groups, analysis of the risk factor (age) in calves revealed no significant difference (p =0.945). In conclusion, this study indicated that Cryptosporidium infection is prevalent in Asella, especially in calves less than 4 month age, poor body condition, female and those calves managed intensively.

Key words: Asella · Calves · Cryptosporidium · Ethiopia · Prevalence · Risk Factors,

INTRODUCTION

Cryptosporidiosis is an enteric protozoan disease caused by Cryptosporidium species of medical and veterinary significance worldwide and its fecal-oral transmition by ingestion[1, 2] The close association of humans and livestock as well as the ability of runoff from animal production to contaminate water supplies represents an ever-present risk of human infection [3, 4].

Although more than twenty species of Cryptosporidium parasite have been present on the basis of the animal hosts, the most important species of cryptosporidium such as *C. parvum*, *C. andersoni*, *C. baileyi* and *C.meleagridis* have been reported to cause morbidity and outbreaks of disease. Cryptosporidium bovis has been described recently and highly distributed species that infects primarily post-weaned calves [5, 6]. The most significant zonootic threat for humans is from *C. parvum* and *C. meleagridis*. Both the prevalence and the severity of infection mostly increase in immune deficient animals, especially in neonates of some animal species such as ruminants. At the same time, the infection is self-limiting in healthy or immunocompetent individuals. In cattle, *C. parvum* is considered tobe the most common

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entero parasite in calves during the first week of age, frequently as a coincidental infection with other viral, bacterial and parasitic pathogens [7]. Cryptosporidium infection can negatively influence growth rate, feed conversion and milk production leading to a drop in the economic benefits derived from livestock production. Cattle have been implicated as an important source of *C. parvum* in Pasture run off, which is responsible for environmental contamination and human infection either by direct or indirect contact through fecal contamination of food or water for human consumption (Current and Garcia, 1991). In dairy cattle, cryptosporidiosis is an age related diarrheatic disease; mainly *C. parvum* is found in pre-weaned calves [8].

Cryptosporidiosis is part of calf diarrhea syndrome that can cause considerable losses in calves. The diarrheal syndrome has a complex etiopathogenesis, because various infectious agents either in a single or in combination may cause diarrheatic syndrome. In addition, environmental, managemental and nutritional factors may influence the severity and outcome of the disease [9]. Generally, most studies carried out in different countries have found Cryptosporidium to be the most commonly detected agents in calves and young cattle with diarrhea [9].

Despite different zonootic importance reports from different part of Ethiopia suggest that Cryptosporidiosis is endemic in humans and presence of different dairy farm that are highly important for the country economy which managed under various management system (Intensive, extensive and semi-intensive), very few research relating to calf cryptosporidiosis has been carried out in other parts of Ethiopia by Wegayehu*et al.* [10] in central Ethiopia; Dinka and Berhanu [11] in Bishoftu; Alemayehu *et al*. [12] in Haramaya and Rahmeto*et al.*[13] in central Ethiopia, even though there are commonly diarrheic calves at the field. There has been poorly documented previous information on calves' cryptosporidiosis in Asella where dairy farms are back bone of the area's economy. Particularly, no study has been done in Ethiopia combining conventional acid fast staining diagnostic method in cattle. Therefore the Objectives of this study were: To study the prevalence of Cryptosporidium among calves and assess associated risk factors under different management systems in Asella town.

MATERIALS AND METHODS

Study Area: The study was carried out from November 2014 to March 2015 in Asella town, southeastern, Ethiopia dairy farm. Asella town is situated at 60591-80491 N latitude and 380411-400441 E longitude in central Ethiopia, 175 km south east of Addis Ababa. The altitude of the area ranges from 1780-3100 m.a.s.l and characterized by mid subtropical temperature ranging from 10-250c. The area covers 23,674.72km square and topographically the area has highland escarpment, midland and lowland climatic zone. About 37% of the total areas are highland (>2400m.a.s.l), 52% of the total areas are midland (1800-2400m.a.s.l) and 11% of the total areas are lowland (<1800 m.a.s.l). The area receives annual rain fall range from 700-1658mm and the annual humidity of the area ranges from 43-60%. The area has bimodal rain fall occurring from March to April (short rainy season) and from July to October (long rainy season). The area mostly covered with clay type of soil and in rare case black soil [14].

Study Animals: Asella town and the surrounding farming community has a total area of 300.2 square km. Out of this about 208.43 square km (69.4%) of the total area are agricultural land, 40.61 square km (13.5%) of total lands are pastoral land, 6.74 square km (2.3%) forest, 39.34 square km (13.5%) land for construction and 5.08 square km (1.69%) none fertile land. The livestock estimate of the year 2010\11 given by Tiyo Woreda Rural and Agricultural Development indicates that the Woreda has 50347 bovines, 16964 equines, 19453 ovines, 6884 caprine and 22485 poultry. The present study was conducted on both sexes of local, cross and pure breeds of calves which are under age of one year and selected randomly from peasant association in Asella town. The study animals were 384 calves of three breeds (local, cross and pure breeds), both sexes (male and female) and less than 12 month age groups.

Body condition scoring was made according to Morgan *et al.* [15] and recorded as poor, medium or good. Due to the absence of written records, the age of the animal was estimated based on owners' response and also by looking to the dentition pattern of the animals [16]. Based on this the study animals grouped under 12 months are calves. Accordingly, animals from different management system (extensive, semi intensive and intensive management) of smallholder farming was recorded. The sample was also included from clinical and clinically health animals.

Study Design: A cross sectional study type was under taken to determine the prevalence of cryptosporidiosis in bovine animal species by the examination of feacalsamples.The required sample size was calculated according to the formula given by Thrusfield [17].

$$n = \frac{(1.96)^2 p_{exp} (1-p_{exp})}{d^2} = \frac{(1.96)^2 \ 0.5(1-0.5)}{(0.05)^2}$$

Where, n = required sample size, pexp = expected prevalence, d2= desired absolute precision at 95% Confidence interval.

There is no data available on the prevalence of cryptosporidiosis at the study area (Asella). As stated above, the confidence interval chosen is 95% so that d = 5% and the expected prevalence is 50%. By substituting the value, the required sample size was calculated to be 384.

Sample Collection

Sampling Method: Simple random sampling was carried out to select the herds of extensive management, semi intensive and farms of intensive management system. To collect the sample from the rectum of animals a plastic glove was used and the collection was carried out into sterile universal bottles which was labeled accordingly and soon brought from study area to Asella Regional Veterinary Laboratory. Information about the age, sex, body conditions, owner name and date of collection was recorded. After the collection the sample was diagnosed with in 48 hours of collection and the left was preserved by 10% formalin to prevent the decomposition of oocyst and shrinkage of the oocysts for further processing.

Fecal Sample Examination: Cryptosporidium infections were diagnosed by acid-fast (modified Ziehl–Neelsen) staining after concentration of oocyst by sedimentation and making fecal smears from the concentrate. Microscopic investigations were used to check the presence of oocysts in the samples. Differential staining using a modified Ziehl-Neelsen technique was employed to differentiate Cryptosporidium oocysts from other cells and artifacts. The oocysts are stained by acid-fast so it stain and appear pink to red color oocyst against a blue background. A positive sample for Cryptosporidium was placed in the microscope as a control. The procedure was performed as follows: 200mg of feces were putted into a sterile conical flask and 800µl distilled water was added, mixed thoroughly with the applicator stick and stand for 15 min. The samples were filtered using cotton cloth gauze and the sediment was used to make simple smears on labeled slides using a drop of normal saline. The smears were dry by using Bunsen burner, then fixed by dipping six times in 10% methanol and allowed to dry in air. Then, the slides were placed in carbolfuchsin for about 7 min and washed in running tap water. The smears were decolorized in 3% acid alcohol. Then the smear were washed in running tap water, stained in methylene blue for about 7 min, then washed and left to dry. After then, the smears were observed under ×100 microscope objectives lens by adding one drop of oil emersion and oocysts will be identified according to standard methods [18].

Data Analysis: Data was entered and managed in Microsoft Excel program and analysis was done by comparing proportions (prevalence) distribution of positive and negative results using Pearson's chi-square test using SPSS version16.0. The 95% confidence intervals was generated for the prevalences and the result were considered significant when p-value < 0.05 and none significant for p > 0.05. The total prevalence was calculated by dividing the number of cryptosporidium positive animals by the total number of animals examined [19].

RESULTS

The Overall Prevalence of *Cryptosporidium* Oocysts in Faecal Samples with Associated Risk Factor: The overall prevalence of cryptosporidiosis in the study area (Asella) was found to be 24.0% (92/384) with the prevalence of 10.3% (7/68), 24.9% (67/269) and 38.3% (18/47) with in local, cross and exotic breed respectively with a highly significant difference (P = 0.002).There was also statically significant variation in body condition score with (P=0.012) and prevalence of 30.4%, 20.2% and 8.0% in poor, medium and good body condition respectively (Table 1).

In contrast there was no statistical variation (P>0.05) with in age and sex with P=0.945 and P=0.176 respectively. The highest and lowest prevalence of cryptosporidiosis with in sex 26.7% and 20.8% were recorded in female and male respectively while there was no statically significance. A prevalence of 24.8%, 23.8% and 22.9% were also observed in = 4, 5-8 and 9-12 months age groups respectively. Generally the result of cryptosporidiosis with associated host risk factor in Asella town was stated in Table 1 as follows.

Farming system, were also some of the management related risk factors assessed in this study. So that, the present study result indicated that the prevalence of cryptosporidiosis in relation to management system was statically insignificant in the study area (P = 0.148, $\div^2$ = 3.817). Although the prevalence of *cryptosporidium* in intensively managed calves was high (30.1%) as compared to extensively (25.2%) and semi-intensively (19.5%) managed calves as shown in Table 2.

Table 1: The prevalence of cryptosporidiosis with host related risk factors

Risk factor	No. examined	No. of positive	Prevalence	x^2	P-value
Breed					
Local	68	7	10.3		
Cross	269	67	24.9	12.407	0.002
Exotic	47	18	38.3		
Sex					
Female	206	55	26.7	1.822	0.176
Male	178	37	20.8		
Age					
0.945					
≤ 4 month	141	35	24.8	0.113	
5-8 month	160	38	23.8		
9-12 month	83	19	22.9		
Body condition					
Poor	171	52	30.4		
Medium	188	38	20.2	8.849	0.012
Good	25	2	8		
Total	384	92	24.0		

Table 2: Prevalence of cryptosporidiosis in calves with management related risk factors

Risk factor	No. examined	No. of positive	Prevalence(%)	x^2	P-value
Management system					
Extensive	127	32	25.2		
Intensive	93	28	30.1	3.817	0.148
Semi-intensive	164	32	19.5		
Total	384	92	24.0		

DISCUSSIONS

In current study the overall prevalence of Cryptosporidium in calves was 24.0%. The overall prevalence (24.0%) of Cryptosporidium in calves in this study was relatively comparable with some of the previous reports of 23.4% in cattle in Nigeria [20] and 24.20% in diarrheic and 16.60% in non-diarrheic calves in India [21], 23.6% overall prevalence in calve, lamb and kid with 27.8% in calves in Haramaya Ethiopia [12]. The Lower prevalences of 10.6% in central parts of Ethiopia [22], 11.9% in USA in dairy cattle [23], 17.6% in dairy calves in central Ethiopia [13], 17.9% in calves in France [24], 19.2% in three cattle husbandry system in Zambia [25] were reported. On the other hand, there were relatively higher prevalence reports of Cryptosporidium in different countries. 27.9% prevalence in young calves in UK [26], prevelance of 33.5% in cattle in Vietnam [27], Prevalence of 35% in calves in the United States of America [28] and prevalence of 47.9% in neonate bovine in Spain by Castro-Hermida *et al*. [29] were reported. Exceptionally, some of the reports indicated the highest prevalence of 50% in Netherlands [30], 58% in Belgium [31], 70% in USA [32], 80 % in calves in Britain [33] was reported. These differences in the prevalence among countries may be as a result of the difference in the stocking rate and husbandry system of livestock production system of the countries. It may also due to difference in climatic condition and seasonal variation during study. Besides these, variations could also be due to the difference in the susceptibility of the target population that related to age difference and breed of study animals[25].

It was observed that the calves below 4 month of age was found to have cryptosporidiosis, compared to those between 5 to 8 month and 9 to 12 month with a prevalence of 24.8%, 23.8% and 22.9% respectively. Similar observations were also made by Brook *et al.* [26], who reported (Prevalence 27.9%) that calves less than 4 months were 13 times more likely to be infected with Cryptosporidium than older ones. Similarly Alemayehu*et al.*[12] reported that calves under 3 month were at higher risk of infection as compared to the older ones (< 3month

age 35.5%, 3-6 month age 25.7% and > 6 month age 16.7%). Additionally Santin*et al*. [28] in USA (66.7% in 2 weeks and 30.4% in 6 month age claves), Nguyen *et al*. [27] in Veitnem (P = 33.5% in calves < 6 month) and Mehdiazami [34] in Iran (P = 10.5% in <1month age, 9.5% in 1-6 month and 9% in 7-12 month age) were also reported similar observation. In other case Shobhamani [35], Roy *et al.* [36] were also reported higher prevalence of infection among calves less than 6 months of age.

So that the present study indicated that the younger animals were susceptible to infection with cryptosporidiosis as compared to adult animals. The higher prevalence in this age group can be related to the fact that these age groups are susceptible to the disease because of the immature immune system of the animal at this age. Kvac*et al*. [37] explained that the animal is becoming resistant with age due to the development immune system through time as the age of animals increase. However lower age groups were highly susceptible this findings show that there was no statistically significant difference (P > 0.05) between the prevalence of cryptosporidiosis in calves in related to age (P = 0.945). But most published studies indicate that the prevalence was significantly higher in newborn and suckling calves [38], Lefay*et al.* [24] and Castro-hermida*et al.* [29]. Insignificance of this finding could be due to the unique management systems employed mostly by extensive management system, where young calves, irrespective of their age, are raised together with their parents under the same field conditions in most farms of this study area, in related to what is mostly practiced in developed nations where the calves are isolated from the dam. Calves raised in such confinement were found to be more susceptible to Cryptosporidium infection due to poor immunity and easy of oocyst contamination through bucket feeding [29]. Therefore, factors other than age may play an important role in determining the susceptibility of calves to Cryptosporidiosis.

Three different breeds of calves (exotic, cross and local) were examined during present study. Out of 47 exotic (HF) calves examined 18 (38.3%) were found positive for cryptosporidiosis while out of 269 cross calves and 68 local calves examined, 67 (24.9%) and 7(10.3%) were found positive respectively. So that this study indicated that exotic breeds were highly susceptible to cryptosporidiosis as compared to cross and local breed. This may be due to lack of adaptation to climatic condition of the area and difference of immunity related host resistance of this three breed. The variation in prevalence of this three breeds were statistically highly significant (P = 0.002). This study was similar with observations made by Alemayehu *et al*. [12] who recorded higher prevalence of (28%) among Holstein Friesian (HF), 25% in cross breed and 27% in local Ogaden cattle in Haramaya, Ethiopia. Additionally prevelance of 6.77% in HF and 5.88% in jersey breed in India [21], prevelance of 44% in HF, 58% in Ayrshire and 0.1% in jersey breeds in Tanzania [8] were also report similar findings. According to Alemayehu*et al.* [12] the difference in prevalence of cryptosporidiosis with in Holstein, Ogaden and cross breed were statistically non significant.

It was observed that the female calves' prevalence was 26.7% while that of male was 20.8% with 0.176 P-value. This indicates that female calves were found to have cryptosporidiosis, as compared to male. Similarly higher prevalence observations in female than in male were also made by Mallinath*et al*.[21] in India (6% in females and 3.67% in male calves), Ayinmode and Fagbemi [20] in south western Nigeria (38.1% in female and 17.1 in male) with the report of female animals twice as likely to be at risk than the males (OR: 2.147). In this study Cryptosporidium infection related to sex was statistically non significant. This was similar with the previous finding of Rehaman *et al.* [39] and Shobhamani [35].

In this study out of 92 intensively managed calves 32 (30.1%) were found positive while out of 127 extensively managed calves and 164 semi-intensively managed calves, 32 (25.2%) and 32(19.5%) calves were found positive respectively. The management system has no significant (P = 0.148) association on prevalence of infection. This finding was in agreement with previous observation of Swai*et al.* [8] in Tanzania (56% in intensive, 28% in extensive and 16% in semi-intensive management), While Castro-Hermida*et al*. [29] and Geurden*et al.* [25], were also explain that the higher stocking rate enhances the infection since infected calves produce large numbers of oocyst into confined calf house ensuring a high environmental contamination. So that Calves rose in such confinement were found to be more susceptible to Cryptosporidium infection due to poor immunity and easy of oocyst contamination through bucket feeding and easy direct contact between calves. In contrast in the extensively reared and traditional husbandry system, in which calves remain in large outside with dam on pasture, the oocyst are dispersed on a large surface and are exposed to direct sunlight, which reduces the oocyst viability, resulting in a reduced infection [24]. Generally farms that has poor hygiene with dirty and muddy surface, created a favorable

condition for the persistence of Cryptosporidium oocysts on the farms[13]. The association of infection with hygienic deficiencies has also been reported by other studies [40].

The prevalence of cryptosporidiosis in calves with poor, medium and good body condition was 30.4%, 20.2% and 8.0% respectively. There was significant difference in Cryptosporidium infection with in these three body condition score with higher prevalence in calves with poor body condition scores. This can be related to immunity of poor body condition calves as immune status of the animal is decreased. Similarly David *et al.* [41] and Swai*et al*. [42] in Tanzania observed that a poor body condition and diarrhea are the most prominent signs of cryptosporidiosis. Additionally some synergic infection of enter pathogens can result in poor body condition, immuno-compromisation and increase new born calves susceptibility to cryptosporidium infection [24].

CONCLUSION AND RECOMMENDATIONS

Cryptosporidiosis is important calf health problems in the study area. This study indicates that the occurrence of Cryptosporidium in calves, with higher prevalence among calves less than 4 months of age when compared with other age groups. Mostly the disease affects new born calves and calves having poor and medium body condition and exotic breed due to inadequate development of the immune system in new born calves and less immunity in poor body condition and due to stress as result of poor adaptation to climatic condition and lack of natural immunity in exotic breeds. Concerning other risk factors, higher occurrence was observed in intensive management system than in extensive and semi-intensive management system. Since large number of extensive management system was present in the study area considerable contamination to the communal pasture grazing system could be the other factor which favors the transmission of the oocyst. Animals of deferent age and sex group usually graze on communal pasture facilitated easy transmission of this disease. In other case cryptosporidiosis prevalence was high in intensive management system in contrast to extensive management system due to high contact between calves managed under intensive management system in relation to those managed extensively in which oocysts were dispersed on ground and exposed to sun light. In general, the problem due to Cryptosporidium in the study area was given less attention because of its sub clinical nature of the disease especially in adult animals.

Therefore, based on the above conclusions, the following points were recommended: The owner should provide adequate colostrum to new born calves in order to control diarrhea caused by cryptosporidiosis, the owner of calves should be aware to improve the management system and health care of calves, further study should be carried out on identification and molecular characterization of Cryptosporidium species in calves and humans in Asella and analysis of the drinking water sources for the presence of oocysts should be done.

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