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Prevalence of *Cryptosporidium* Infection in Calves Aged less than One Year in Urban and Peri Urban Areas of Gondar Town

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Abstract: A cross sectional study was conducted from October 2014 to March 2015 with the objectives of determining the prevalence of *Cryptosporidium* infection and assessing associated risk factors in calves aged less than a year in urban and peri urban areas of Gondar town. A total of 360 fecal samples were collected and examined by centrifugal-Sheather's floatation and modified Ziehl Nielsen's acid fast staining techniques to detect the presence of the parasite oocysts in the collected fecal samples. Therefore, out of the total 360 faeces examined, 18.6% (67/384) were positive for *Cryptosporidium* oocyst. Age, sex, breed, herd size, geographical origin, hygienic status, body condition, water and feed type, contact with other animals and fecal consistency of the calves were taken as the determinant factors to assess their significances with the occurrences of *Cryptosporidium* infection. Significant associations were evidenced between cryptosporidial infection in calves with fecal consistency, aged groups, hygienic status and contact with other animals, drinking water source and feed type. The occurrence of *Cryptosporidium* infection was relatively higher in calves with: poor body condition (19.1%), herd size greater than 20 animals (20.3%) and in extensive production system (23.7%). However, there was no a significant interaction ($P > 0.05$) between these factors and the prevalence of parasitic infection. Moreover, prevalence of *Cryptosporidium* infection in peri urban areas (21.3%) was higher than urban (14.8%), but it was not statistically significant ($P > 0.05$). Generally, the present study figured out that cryptosporidiosis is prevalent in urban and peri areas of Gondar town. Hence, a continuous education and extension services are recommended in order to adopt integral approach involving good hygienic practice, decrease contact with animals and use of anticryptosporidial drugs.

Key words: *Cryptosporidium* Infection · Prevalence · Calves · Gondar

INTRODUCTION

Cryptosporidiosis is one of the infectious diseases caused by protozoan parasites under the genus *Cryptosporidium*, in the phylum Apicomplexa. It is an acute or chronic enteric disease which is considered to be a serious disease of all animals specially calves and lambs from the economic point of view; causing morbidity and mortality, impairing rate of weight gain in feed lot and milk production in dairy cattle [1]. Parasites under the genus *Cryptosporidium* are ubiquitous enteric protozoan parasites infecting a wide range of hosts including mammals, birds, reptiles and fish [2]. It is intra cellular, extra-cytoplasmic coccidian parasites which develop and multiply in the epithelial cells of the gastro-intestinal or respiratory tract of a wide range of vertebrate hosts [3].

According to [4], *Cryptosporidium* species are broad spectrum protozoan in which about sixteen species are validated to be the cause of infection: (*Cryptosporidium hominis*, *C. parvum C. suis*, *C. felis*, *C. andersoni*, *C. canis*, *C. muris*, *C. bovis*, *C. wrairi*) in mammals, (*C. meleagridis*, *C. baileyi*, *C. galli*) in avian, (*C. serpentis*, *C. saurophilum*) in reptiles and (*C. scophthalmi*, *C. molnari*) in fish. *Cryptosporidium parvum* is the major species seen in human and animals. Anthroponotic *C. parvum* type I (*C. hominis*) is found in humans and *C. parvum* type II (*C. bovis*) is found in humans and animals zootomically.

To date, seven species (i.e. *C. andersoni*, *C. bovis*, *C. felis*, *C. hominis*, *C. parvum*, *C. ryanae* and *C. suis*) and two genotypes of *Cryptosporidium* (i.e. "pig genotype II" and a new "*C. suis*-like genotype") have been recorded in

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cattle [5]. Abu-Madi *et al.* [6] indicated that cryptosporidiosis can be transmitted from human to human or calf to calf (anthroponotic transmission) or from animal to human (Zoonotic transmission). Ruminants often have been implicated as a major source of human cryptosporidiosis [7]. Infections are usually transmitted via the fecal-oral route, following direct or indirect contact with infective stages of the parasite (the fully sporulatedoocysts when excreted) [8]. Although infection is often self-limiting in immuno-competent individuals, it can become sever and chronic in young and immuno-compromised or suppressed individuals [9].

According to Brook *et al.* [1], the key risk factors for higher prevalence of cryptosporidiosis in calves include: being young aged, a thick depth of bedding and poor sanitation. [6] also indicated the key risk factors of human cryptosporidiosis as contact with individuals with diarrhea, swimming in public pools, travel to developing countries and, importantly, direct contact with animals.

Clinical cryptosporidiosis is frequently not diagnosed, yet it has been incriminated as an important cause of diarrhea in neonates [10]. Clinically the disease is characterized by anorexia and diarrhea, which may result in poor growth rate. The severity of clinical disease may be associated with animals' immune and nutritional status [11]. According to Nasir *et al.* [12] although calves of 1-3 week old were seem to be more susceptible; cryptosporidium species has also been found in cattle of all age group. Variety of methods is available for detection of cryptosporidium. These include microscopic, immunological and molecular techniques. Oocysts can be demonstrated using Ziehl-Nelsen's acid fast stained fecal smears in which the sporozoites appear as bright red granules [13].

Information about the prevalence and associated risk factors to *Cryptosporidium* infection is an essential points to design and implement control strategies against cryptosporidiosis. However, there is limited information in Ethiopia on the status of *Cryptosporidium* infection and cryptosporidiosis in calves [14, 15]. Therefore, the objectives of this study were:

- To determine the prevalence of *Cryptosporidium* infection in calves less than a year aged in the study area.
- To asses risk factors associated with the occurrence of the infection in calves

MATERIALS AND METHODS

Study Area: This cross-sectional study was conducted in and around Gondar town which is located in Northwestern part of Ethiopia at about 727 km far from Addis Abeba. Its altitude is 2200 m.a.s.l. which lies at mid agro-climate zone. It receives a bimodal rain fall: the average annual rain fall is about 1172 mm with 19.7°C annual average temperature. Based on the 2013 census of animal production and extension sector of Gondar town agricultural bureau, the livestock populations in and around Gondar town are as follows: 78,123 indigenous breed of cattle, 2330 cross breed cattle, 982 equines, 20567 sheep, 21515 goat, 68408 poultry and 6909 bee hives.

Study Population: The study animals were both cross and indigenous breed calves aged less than one year. During sampling period, their history, age, sex, breed, feeding practice, water source, current health status, herd size, hygienic condition, breed and management system were recorded using data gathering sheet and personnel observation. The study was carried out on a total 360 calves.

Sample Size Determination and Sampling Technique: The sampling technique encountered was simple random sampling method that the dairy farms in the study areas were listed by the farms owner name in a sheet and then the required farms were selected randomly from the sample frame prepared in the sheet. As far as the sample size was achieved all calves in the selected farms was taken as sample (1-6 calves/farm). The sampling was determined by the formula given by [16]. Since there was no any study done previously on the study area, the expected prevalence was 50% with the confidence interval of 95% and 5% desired absolute precision.

$$n = \frac{(1.96)^2\ p(1-p)}{d^2}$$

where,
n= required sample size, P= expected prevalence, d= desired absolute precision

Therefore, the total required sample size was 384. However, only 360 calves were selected from dairy calves aged less than one year since it was difficult to get all expected samples due to logistics problem.

Study Design: A cross-sectional study was used to determine the prevalence and risk factors of *Cryptosporidium* infection in calves less than one year old through collecting fecal samples from urban and per urban areas of Gondar town.

Parasitological Examination Method: Fresh fecal samples were collected from each calf rectum using disposable plastic gloves. The sample was then put in a separate plastic container to transport with cold box to laboratory on the same day of collection and preserved at 4°C until processing. At the time of sampling, consistency of the feces (normal, diarrheic), age, sex, breed and production system, drinking water source, feeding type, contact with other animals, body condition and hygiene of the calves themselves and their house was recorded on each calf record format. A qualitative fecal examination technique was used to recover *Cryptosporidium* oocyst from the collected faecal samples. The samples were processed using centrifugal fecal floatation (using concentrated sugar solution or Sheather's sugar solution) to separate and concentrate the oocysts of *Cryptosporidium* species. Moreover, each sample was also subjected to microscopic examination of fecal smear using Modified Ziehl Nelson's acid fast staining technique.

Data Management and Analysis: The data obtained from the owners and laboratory results (centrifugal Sheather's floatation technique and Modified Ziehl Nelsons' acid fast staining technique) were recorded on Microsoft excel work sheet. Then analysis was made by SPSS version 16 statistical software to summarize the raw data. Percentage and frequency tables were used to show the prevalence of infection rate. Chi-square test was used to determine the association between different variables and occurrence of *Cryptosporidium* infection. Fisher's exact test was also used when the numbers with in categories were too small for the Chi-square test. A statistically significant association between the variables and the infections was considered to exist if the P-value is less than 0.05.

RESULTS

Prevalence of Cryptosporidium Infection: Out of the total 360 fecal samples collected from calves with aged under a year, 67 were positive for cryptosporidium infection with the overall prevalence of 18.6%. Different potential risk factors were considered in relation with the prevalence of *Cryptosporidium* infection in calves. Origin, herd size, sex, breed type, age, body condition, production system, feed type, water source, hygienic status, faecal consistency and contact with other animals were among the potential risk factors considered.

Origin: The overall prevalence of *Cryptosporidium* infection in calves according to their origin was 21.3% in peri-urban and 14.8% in urban. However, the difference was not statistically significant ($x^2 = 2.483$, P = 0.115).

Table 1: Prevalence of *Cryptosporidium* infection based on calves origin

Origin	No. examined animals	Positive cases	Prevalence (%)
Urban	149	22	14.8
Peri-urban	211	45	21.3
Total	360	67	18.7

$x^2 = 2.483$; P = 0.115

Herd size: Regarding herd size, the occurrence of *Cryptosporidium* infection in calves was higher (20.3%) in a herd greater than 20 animals than in a herd constitute less than or equal to 10 animals (17.7%) and 11- 20 animals (19.3%). But there was no significant difference ($x^2 = 0.019$, P = 0.891) among herd size with the occurrence rate of infection.

Table 2: Association of *Cryptosporidium* infection in calves with herd size

Herd size	No. examined animals	N. positive cases	Prevalence (%)
<10 animals	175	31	17.7
11-20 animals	140	27	19.3
> 20 animals	45	9	20.3
Total	360	67	18.6

$x^2 = 0.019$, P = 0.891

Breed: Although breed was not showed a significant ($x^2 = 0.585$, P = 0.444) effect on the prevalence of *Cryptosporidium* infection, higher infection rate (21.3%) was recorded in cross breed calves than local breed calves (17.7%).

Table 3: Overall prevalence of *Cryptosporidium* infection in relation with calf breed

Breed type	No. examined animals	N. positive cases	Prevalence (%)
Indigenous breed	171	48	17.7
Cross breed	89	19	21.3
Total	360	67	18.6

$x^2 = 0.585$, P = 0.444

Sex: Sex-wise prevalence of *Cryptosporidium* infection in calves was found to be 19.1% in female and 18.2% in males. But it was not significantly ($x^2 = 0.054$, P = 0.817) varied between sex groups.

Table 4: Sex-wise prevalence of *Cryptosporidium* infection in sampled calves

Sex	No. examined animals	N. positive cases	Prevalence (%)
Male	162	31	19.1
Female	198	36	18.2
Total	360	67	18.6

$x^2 = 0.054$, P = 0.817

Age-Wise Prevalence of *Cryptosporidium* infection: Age-wise prevalence of cryptosporidium infection was found to be 28.4% in calves with less than six months of age and 15.6% in calves with six to twelve months of age. It was significantly ($x^2 = 6.605$, P = 0.010) varied between the two age groups.

Table 5: Age-wise prevalence of *Cryptosporidium* infection in calves

Age	Examined animals	N. positive cases	Prevalence (%)
Less than 6 month	81	23	28.4
6-12 months	279	44	15.6
Total	360	67	18.6

x^2=6.605, P=0.010

Body Condition Score: A significant ($x^2 = 0.019$, P = 0.891) association was not observed between body condition score and overall prevalence of *Cryptosporidium* infection in calves. However, the overall prevalence of *Cryptosporidium* infection was slightly higher in poor condition score calves (19.1%) than in good score calves (18.5%).

Table 6: Overall prevalence of *Cryptosporidium* infection with body condition score

Body condition	No. examined animals	N. positive cases	Prevalence (%)
Good	271	50	18.5
Poor	89	17	19.1
Total	369	67	18.6

$x^2 = 0.019$, P = 0.891

Production System: Higher prevalence (23.7%) of infection was observed in calves managed extensively than in calves with intensive and semi intensive production system. But it showed insignificant ($x^2 = 0.722$, P = 0.692) variation among production systems.

Table 7: Prevalence of *Cryptosporidium* infection in relation with production system

Production system	No. examined animals	N. positive cases	Prevalence (%)
Intensive	100	18	18
Semi-intensive	222	40	18
Extensive	38	9	23.7
Total	360	67	18.6

$x^2 = 0.722$, P = 0.692

Feed Type: In the present study, feed type was considered as a risk factor for the occurrence of *Cryptosporidium* infection. Accordingly, animals that fed on milk (non-weaned calves) had higher cryptosporidium infection (23.4%) than calves that fed on non-milk feed (weaned calves) (11.9%). It was significantly varied ($x^2 = 8.641$, P = 0.013) between the two feed types.

Table 8: Overall prevalence of *Cryptosporidium* infection in relation with feed type

Feed source	No. examined	positive cases	Prevalence (%)
Pasture only	151	18	11.9
Milk and pasture	209	49	23.4
Total	360	67	18.6

$x^2 = 8.641$, P = 0.013

Water Source: A significant association ($x^2 = 12.43$, P = 0.002) was observed between water source and prevalence of *Cryptosporidium* infection in calves in this attempt. Calves which had been getting river water had higher overall prevalence of *Cryptosporidium* infection (29.4%) than those calves that had pipe water (10.9%).

Table 9: Association of water source with the occurrence of Cryptosporidium infection in calves

Water source	No. examined animals	N. positive cases	Prevalence (%)
Pipe	165	18	10.9
River	195	49	25.13
Total	360	67	18.6

$x^2 = 12.43$, P = 0.002

Hygienic Status of Calves and Their House: Regarding hygienic status of calves and their house, out of 360 animals 296 had good hygiene and 64 had poor hygiene at the time of sampling. Out of 64 calves with poor hygiene, 22 (34.4%) were positive for *Cryptosporidium* oocysts. Among the 296 calves with good hygiene, 45 (15.2%) were positive for *Cryptosporidium* infection. The difference was between hygienic status for *Cryptosporidium* infection was statistically significant ($x^2 = 12.77$, P = 0.000).

Table 10: Association of *Cryptosporidium* infection in calves' with hygiene status

Hygiene	No. examined animals	N. positive cases	Prevalence (%)
Good	296	45	15.2
Poor	64	22 3	4.4
Total	360	67	18.6

$x^2 = 12.77$, P <0.001

Faecal consistency: Of 360 calves examined, 62 had diarrheic and 298 had non-diarrheic faecal consistency. Among 62 calves with diarrheic faeces, 22 (35.5%) were

positive for *Cryptosporidium* infection. Similarly, out of 298 calves with non-diarrheic faecal matter, 45 (15.1%) were positive for *Cryptosporidium* oocysts. The difference between faecal consistency being positive for *Cryptosporidium* infection was significant ($x^2 = 14.08$, $P < 0.001$).

Table 11: Prevalence of *Cryptosporidium* infection in relation with faecal consistency

Diarrhea	No. examined animals	N. positive cases	Prevalence (%)
Non diarrheic	298	45	15.1
Diarrheic	62	22	35.5
Total	360	67	18.6

$x^2 = 14.08$, $P < 0.001$

Contact with Other Domestic Animals: In this survey, direct contact with other domestic animals (like pets, poultry, sheep, goat, adult cattle and equines) was considered as a factor for *Cryptosporidium* infection. Accordingly, calves that had direct contact with other domestic animals had a significant ($x^2 = 4.384$, $P = 0.036$) higher infection rate (61/296, 20.6%) than those calves which had no any contact with other animals (6/64, 9.4%).

Table 12: Rate of *Cryptosporidium* infection in calves in relation with contact with other animals

Contact	No. examined animals	N. positive cases	Prevalence (%)
No contact with other animals	64	6	9.4
Contact with other animals	296	61	20.6
Total	360	67	18.6

$x^2 = 4.384$, $P = 0.036$

DISCUSSION

The overall prevalence of *Cryptosporidium* infection in calves under a year in the present study was found to be 18.6 % (67/360). This is in agreement with the work of Abebe, Wssene and Kumsa [14] who reported *Cryptosporidium* infection at a rate of 17.6% in dairy calves in Central Ethiopia. Similarly, Lefy, Naciri and Vitovec [17] from France and Geurden *et al.* [18] from Zambia reported a comparable infection rate in dairy calves at 17.9 % and 19.2 %, respectively. However, my finding is higher than the report of Fayer and Xiao [19] in USA at a rate of 11.9%. On the other hand, Santin *et al.* [20] in USA, Nguyen *et al.* [21] in Vietnam, Brook *et al.* [1] in UK, Dilrukshi *et al*. [22] in Sirlanka and Castro-Hermida, Gonzalez-Losadaand Ares-Mazas [23] in Spain reported relatively higher infection rate in calves at a rate of 35.5%, 33.5%, 27.9%, 28.5% and 47.9%, respectively. This difference might be due to the geographic difference, study design and period as well as season of the year when the study was conducted.

Production system had shown insignificant association with *Cryptosporidium* infection in calves in the present study. However, the overall prevalence of *Cryptosporidium* infection was higher in those calves managed under extensive system than other husbandry systems. This suggests that extensive husbandry system could increase the exposure rate of animals to *Cryptosporidium* infection. Moreover, the occurrence of cryptosporidium infection in calves was relatively higher in peri-urban calves than urban calves.

Age-wise prevalence of *Cryptosporidium* infection in this attempt was significantly higher in calves whose age less than six months than in those calves whose age six to twelve months. According to Geurden *et al.* [18], age has a significant effect on the occurrence of *Cryptosporidium* infection in animals. Similarly, Dilrukshi *et al*.[22] stated that age appears to be an important factor that influences the occurrence of *Cryptosporidium* infection. These explanation supports the present finding in which higher prevalence rate recorded in calves whose age less than six months. This might be attributed to the fact that the immature immune system of calves at this age level. Similarly, [1, 23], stated that calves under 4 months of age are more susceptible to cryptosporidium infection than older ones. This is also reasonably supported by [24], who explained that resistant to infection could be developed with age due to immune development through time. Therefore, though *Cryptosporidium* infection can be observed in all age groups of animals, the prevalence of this parasite infection is larger in young calves [7].

A strong association was observed between hygienic status of calves and their house and the occurrence of *Cryptosporidium* oocysts in faeces of examined calves. Our finding supports the report of Abebe, Wsseneand Kumsa [14] who described the significant association of the Cryptosporidium infection with hygienic status of dairy animals and their farms. This might be due to that dirty and muddy farms could presumably create a favorable condition for the presence of *Cryptosporidium* oocyst on the farms. This favors the exposure rate of calves with poor hygienic status.

Feed types of calves had showed significant influence on the occurrence of *Cryptosporidium* infection in calves. Accordingly, the occurrence of *Cryptosporidium* infection in calves that fed on milk was higher than in calves that fed on pasture only in the present survey. Moreover, significant interaction was

observed in the prevalence of *Cryptosporidium* oocysts shedding with faecal consistency of calves where diarrheic animals were shed *Cryptosporidium* oocysts more frequently than those calves which released normal and soft faecal matter. This is in accordance with Lise *et al.* [25] who reported a strong association between *Cryptosporidium* oocyst shedding and calf diarrhea. They also stated that infected calves were five times more diarrheic than non-infected calves. It appears, therefore, that *Cryptosporidium* is the entero pathogen which strongly associated with diarrhea. This is also supported by Causape *et al*. [26] who reported significant interaction between the occurrence diarrhoea and *Cryptosporidium* infection.

CONCLUSION

This study indicated that *Cryptosporidium* infection is prevalent in calves in the study area. The overall prevalence of *Cryptosporidium* infection in calves originated from urban and peri-urban areas was found to 18.6%. Hygienic status, drinking water source, feed type, contact of calves with other animals and age of calves were the identified potential risk factors which had significant association with the occurrence of *Cryptosporidium* infection. Therefore, based on the result, we can conclude that infected calves could be a reservoir for *Cryptosporidium* infection for humans and other animals. Therefore, based on the above conclusion the following recommendations are forwarded:

- Awareness creation should be practiced for the community about public health and economic significances of cryptosporidiosis and about the proper care to be taken
- Awareness creation for veterinarians and para-veterinarians, construction of clinic and supply of adequate drugs and facilities are also necessary.
- Eventhogh cryptosporidium infection is important problem of calf in the study area, there has not been any study showing the problem still; therefore, further studding involving different risk factors should be conducted on cryptosporidium infection.

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