

Epidemiology and Intensity of *Cryptosporidium* Infection in Cattle and Sheep in Selected Areas of Central Oromia, Ethiopia

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Abstract

Back ground: Cryptosporidiosis is a disease caused by a variety of *Cryptosporidium* species and causes gastrointestinal illness in a wide variety of mammals including humans, cattle, sheep, goats, pigs and horses worldwide.

Methods: A cross sectional study was conducted from December 2021 to July 2022 to estimate the prevalence, identify associated risk factors and estimate infection intensity of the parasite in cattle and sheep in Central Oromia. Fecal flotation sheathers' solution and modified acid fast techniques were used to identify *Cryptosporidium* oocysts from fecal samples.

Results and discussion: Out of 687 fecal samples examined, 182 (34.2%) cattle and 30(19.4%) sheep were found to be infected with *Cryptosporidium*. There was a significant difference ($P < 0.05$) in the *Cryptosporidium* infection between age groups of cattle, with higher prevalence in young (43.4%) than adult age group (28.4%). Similarly, there was a significant difference ($P < 0.05$) in *Cryptosporidium* infection among study sites. There was a considerable difference in *Cryptosporidium* infection between production systems, with higher prevalence in the intensive production system (38.7%) than the extensive system (21.6%). There was also a significant difference in the intensity of infection of *Cryptosporidium* ($P = 0.000$) between the age groups of cattle, with more burden of illness in the young age category than the adult age group. Body condition was found to be the only risk factor associated with *Cryptosporidium* infection in sheep.

Conclusion: The present study indicated that *Cryptosporidium* is widely distributed in cattle and sheep in the study areas. Many adult animals were infected with this parasite, meaning that adult animals could also play a significant role in parasite transmission. The majority of *Cryptosporidium* infections in both cattle and sheep were mild.

Keywords: *Cryptosporidium* • Central Oromia • Intensity of infection • Risk factors • Prevalence

Introduction

Gastrointestinal parasites are considered as one of the most significant constraints in livestock sector. Damages inflicted to the health and productivity includes loss in body weight, poor reproductive performance, digestive disturbances, and emaciation for longer period [1]. It has been established that parasitic infections result in considerable losses in milk production in cattle [2]. Livestock carry large numbers of protozoa in their stomachs and intestines, the vast majority of which are entirely harmless. Some species of protozoa, however, are significant as causes of disease in domestic cattle, sheep and poultry, or because of their potential for zoonotic transmission [3].

Cryptosporidiosis is caused by a protozoan parasite of the genus *Cryptosporidium*, family of Cryptosporididae, Order Eucoccidiorida, class of Coccidia and phylum Apicomplexa. The parasite infects epithelial cells in the microvillus of gastrointestinal tract of all classes of vertebrates and causes

severe chronic and even fatal diarrhea with mal absorption and dehydration [4].

Currently, there are 26 *Cryptosporidium* species and more than 70 genotypes that are recognized as valid on the basis of morphological, biological and molecular data. The hosts ranges and pathogenicity is species variable. Among the species, *Cryptosporidium parvum* is the most common species of medical and veterinary importance. Most people and animals infected with *C. parvum* develop immunity and recover from that infection. However, the disease is persistent and life threatening if there is immunologic impairment [5].

Cryptosporidiosis has a worldwide distribution. It has been documented in people and animals in 95 countries [6]. Most of the published reports were from Europe and little is known about the prevalence of ruminants' Cryptosporidiosis in Africa. Study reports on the occurrence of *Cryptosporidium* in ruminants are also very rare in Ethiopia and some of the prevalence reports range from 7.2% to 18.6% [7-10]. A wide-ranging study conducted in nine regions of the country reported an overall prevalence of 2.3% [11] while a study in eastern region reported a prevalence of 27.8% [12]. Molecular characterization of *Cryptosporidium* isolates from nine regions and central part of the country confirmed the existence of four species: *C. parvum*, *C. andersoni*, *C. bovis* and *C. ryanae* in the Ethiopian cattle [11,12]. This indicates that there is few information on epidemiology, seasonal occurrence and intensity of *Cryptosporidium* infection in cattle and sheep in the study areas. Therefore, this study was initiated to estimate the prevalence of *Cryptosporidium* infections in cattle and sheep, to identify risk factors associated with the occurrence of the parasite and to estimate the intensity/burden of infections by the parasite in cattle and sheep.

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Materials and Methods

Description of study areas

The study was conducted in three selected areas of central Ethiopia namely Bishoftu (Mid land), Adama (Low land) and Holeta (High land) (Figure 1). Cattle and sheep managed under both intensive (Dairy farms) and extensive productions systems were considered for the study.

Bishoftu town is located at 9°N latitude and 40°E longitudes, 47 km south-east of Addis Ababa, at an elevation of 1850 meter above sea level. A bimodal rainfall pattern exists in the area, with a short rainy season from March to May and a longer wet season from June to September. It has an annual rainfall of 866 mm of which 84% is in the long rainy season and the remaining in the short rainy season. The dry season extends from October to February. The area's average annual maximum and lowest temperatures are 26°C and 14°C, respectively, with a 61.3% relative humidity [13]. Farmers in the Bishoftu and its surroundings use mixed agricultural and livestock production. Furthermore, Bishoftu and its surroundings provide a diverse range of agro ecologies that are typical of the country. Different plant and animal species live in these agro-climatic zones. The livestock population on the basis of species is estimated to be 160,697 cattle; 22,181 sheep; 37,510 goats; 1,660 equines and 191,380 poultry [14].

Holeta is a town located in West Shoa Zone, Oromia region, Ethiopia, at a distance of 35 Km from Addis Ababa, lying between elevations of 2,320 and 2,460 meters above sea level. The average rainfall in Holeta is 1,367 mm and the mean temperature varies from 12.3 to 15.9 °C with a 9°15' N and longitude of 38°25'- 38°45' E. Human population in 2015 was estimated to be 57,828 with an average of 6.7 members per household. The area gets annual rain fall of 834-1300 mm and the annual temperature of 11-22°C. Rainy season occurs with bimodal distribution 70% of which occurs during the main rainy season (June to September) and 30% during the small rainy season (February to April) and relative humidity of 50.4% [13]. The main economic activity is agriculture with several crops cultivated in the area. Farming of livestock is rising and contributes to the development of the economy of the area as well. The town obtains grain products, livestock supply, natural resources and labor from surrounding areas and manufacturing and commercial products from Addis Ababa. The total cattle population of the study area is estimated to be 175,741, out of which 172,769 (98.3%) heads of cattle are local breeds and 2972 (1.7%) are crosses kept under extensive and semi intensive management systems, and the remaining are kept in intensive management system. Dairy farm is carried out in the area both in large scale dairy production system for commercial purpose and in smallholder farming system [15].

Adama is a city is located 95 km south eastern Addis Ababa 39.17°N and 8.33°E with an altitude of 1570 meter above sea level, latitude 8.31°N and 39.16°E longitude. Adama is situated in the well-known East African rift valley. It has annual rain fall temperature ranging from 400 mm-800 mm and

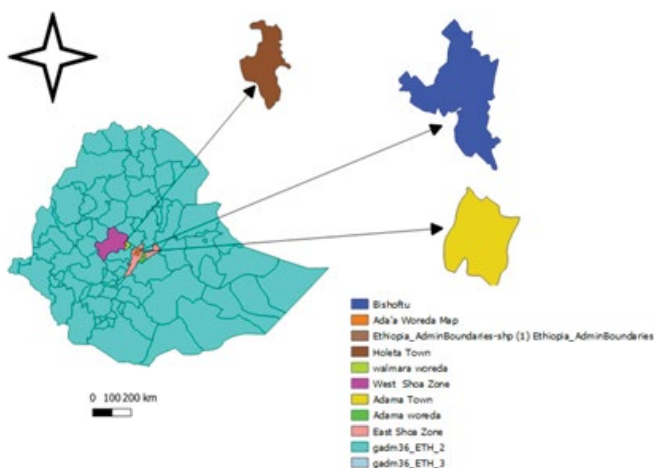


Figure 1. Map showing the study areas.

13.9°C-27.7°C, respectively [13]. The town is one of the most populous from the regional state and is located at an important multidirectional trade route. The numbers of livestock on the bases of species are 70, 662 cattle; 36, 142 sheep; 42, 968 goats; 31, 905 equines; 42 camels and 195, 155 poultry [16].

Study animals

Cattle of different breeds (local, cross and exotic) and sheep were the study population of this study. Fecal samples were collected from five dairy farms (three from Bishoftu, one from Holeta and one from Adama town) and from local cattle and sheep from three veterinary clinics. Production systems, animal species, breed, age, sex, study sites/farms, agro ecology, season of the year, body condition of the animals and fecal consistency were recorded during sampling. The study animals were categorized in to young and adult (for cattle, young refers to less two than years of age; adult is more than two years; for sheep young refers to less than one year and adult is more than one year) according to Jamal G, et al. [17]. The body condition of cattle and sheep was categorized as poor, medium and good respectively as per the guide lines by Klopčič M, et al. [18] and Russel A, et al. [19].

Ethics approval

The approval on animal handling ethics was received before the commencement of the study. All the animal handling and sample collection methods were performed in accordance with the Addis Ababa University College of Veterinary Medicine and agriculture Research Ethics (AAU-CVMA-REC) and animal welfare guide for the care and use of animals (Ref no. VM/ERC/28/03/14/2022).

Study design and sample size determination

A cross-sectional study design was employed for the study. Cluster sampling technique was used for the study involving dairy farms. Whereas, purposive sampling technique was used for collection of fecal samples from veterinary clinics. The desired sample size for the study was calculated using the formula given by [20] with 95% confidence interval and 5% absolute precision.

$$N = \frac{1.96^2 P_{exp} (1 - P_{exp})}{d^2}$$

Where, P_{exp}=expected prevalence; d=absolute precision (5%); N=sample size.

Considering 15.8% expected prevalence of intestinal protozoal infections in cattle [9] in central Ethiopia, the sample size for one study animal species was 204. Since we considered two animal species (cattle and sheep), the total sample size was calculated to be 204 × 2=408. However, we increased the sample size to 687 samples. Since the number of sheep coming to the veterinary clinics was few compared to cattle, we collected fecal samples from only 155 sheep and 532 samples from cattle.

Sample collection and laboratory analysis

Coprological examination: About 10gram of fresh fecal samples was collected from cattle and sheep directly from the rectum using sterile disposable gloves. The samples were placed in labeled universal bottles, preserved in 10% formalin with the ratio of 1 gram of feces to 3 ml of formalin to prevent *Cryptosporidium* oocysts from desiccation, and transported in ice box to the parasitology laboratory of Addis Ababa University, College of Veterinary Medicine and agriculture for laboratory analysis. Fecal flotation using concentrated sugar solution (sheathers' solution) and Modified acid fast techniques were used to detect the oocysts of *Cryptosporidium* [8,10]. A sample was considered positive for *Cryptosporidium* species if an oocyst of correct morphology was detected, i.e. optical properties, internal structure, size and shape as described by Fayer.

Determination of intensity of *Cryptosporidium* infection: The average number of oocysts in 10 randomly selected fields (oocyst per-field) of 100X magnification was used to estimate the intensity of *Cryptosporidium* oocysts. The intensity of oocyst was graded as: low (1-5 oocyst), medium (6-10 oocyst), and high (above 10 oocysts).

Data management and statistical analysis

The statistical analysis was performed using SPSS 26 software. Chi square test and Mann-Whitney Test of non-parametric values were the statistical tests utilized to analyze the data. Logistic regression analysis was also used to identify the potential risk factors associated with the occurrence of intestinal protozoal parasites at a desired precision level of 5% and confidence interval of 95%. Significant difference was considered when $P < 0.05$.

Results

Cryptosporidium infections in cattle and sheep

Out of 687 fecal samples examined by coprology, 212 (30.9%) of cattle and sheep were found to be infected with *Cryptosporidium*. Statistically, there was a significant difference ($P < 0.05$) in *Cryptosporidium* infection between the study animals with higher prevalence in cattle (34.2%) than sheep (19.4%) (Table 1).

***Cryptosporidium* infections in cattle:** Out of 532 fecal samples examined by coprology, 182 cattle (34.2%) were found to be positive for *Cryptosporidium* parasite. Univariate logistic regression analysis indicated that there was a significant difference ($P < 0.05$) in *Cryptosporidium* infections between age groups; among the different body conditions; among study sites/farms, between seasons; among agro - ecologies and between production systems (Table 2). However, in multivariate logistic regression analysis of the data, only three variables namely age of the animals, the study sites/farms and the production systems were found to be potential risk factors associated with the occurrence of *Cryptosporidium* infections in cattle (Table 3).

***Cryptosporidium* infections in sheep:** From a total of 155 fecal samples examined through coprology, 30 sheep (19.4%) were infected with *Cryptosporidium*. Body condition was found to be the only risk factor associated with *Cryptosporidium* infection in sheep (Table 4).

Infection intensity/burden of *Cryptosporidium* parasite

The oocysts of *Cryptosporidium* were detected and semi quantitatively counted under the microscope based on their morphology (Figure 4). The majority of *Cryptosporidium* infections in both cattle and sheep were mild. Low, Medium and high infection intensity in cattle was 37.4%, 26.9% and 33% respectively while the corresponding infection intensity in sheep was 60%, 20% and 23% respectively (Figures 2 and 3).

Age related intensity of *Cryptosporidium* infection

There was a significant difference in the intensity of infection of *Cryptosporidium* ($P = 0.000$) between the age groups of cattle with more burden of infection in the young age category than the adult age group (Table 5). However, there was no significant difference ($P > 0.05$) in intensity of infection between the age groups in sheep.

Discussion

The prevalence of *Cryptosporidium* in cattle in the current study was 34.2%. This finding is similar to the report of (34.1%) from Kafre-Sheikh province and (28.2%) from Mukure district of Kenya. However, the present record is lower than the finding of who reported 64% prevalence from Brazil.

Our finding however, was greater than the reports of authors from different regions of Ethiopia: (13%) from southern Ethiopia; (18.6%) from North West

Ethiopia; [8] (13.6%) from Bishoftu, Ethiopia; [9] (15.8%) from central Ethiopia; (7.8%) from North Shewa and [10] (18.6%) from Addis Ababa, Ethiopia. The differences in the prevalence reports from different study areas could be attributed to the variation in climatic conditions, husbandry practices, the study methodologies employed and the age of the target population for the studies.

There was a significant difference in *Cryptosporidium* infections in cattle between age groups of the animals with higher prevalence in young age group (43.4%) than adult age group (28.4%). This finding agrees with the reports of 2018; who reported high prevalence in young than adult age group. This could be related to the underdeveloped immune system of young age group which results in their higher exposure to *Cryptosporidium* infection. However, the present result disagrees with the findings of and who reported lack of significant difference between the young and adult age groups. The present prevalence in adult age category indicates that adult cattle could shed oocysts of *Cryptosporidium* and play a significant role in parasite transmission through environmental contamination. This is supported by who explained that adult cattle have a role in the outbreak of cryptosporidiosis in calves and humans, even though healthy calves under one month of age are the main carriers of *Cryptosporidium* parasite.

Similarly, there was a significant difference in *Cryptosporidium* infections in cattle among the study sites/dairy farms with higher prevalence in ARPC dairy farm (55.6%) than other dairy farms. There was also a significant difference in the infections between the two production systems with higher prevalence in intensive system (38.7%) than extensive production system (21.6%). This finding agrees with the report of who registered a higher prevalence (42.8%) in intensively managed cattle than in extensively managed cattle. Our finding also agrees with the report of who recorded higher prevalence of *Cryptosporidium* infections (21.4%) in intensively managed cattle than those under the extensive system (11.2%). This difference in prevalence might be due to the difference in management systems in which the extensively reared cattle remain in large outdoor areas, reducing contact and oocyst contamination. In addition, cattle in extensive management systems have lower exposure to infection 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.

The prevalence of *Cryptosporidium* infection in sheep was 19.4%. This finding is greater than the reports of (1.4%) from France; (11%) from Algeria; (5.1%) from Greece; (10.1%) from Poland; (11.3%) from Iran and (10.1%) from Italy.

Our finding however, is lower than the prevalence reports of (29.06%) from Greece; (28.7%) from Nigeria; (28.5%) from China and (30.6%) from California, USA. The low prevalence in sheep in this study could be due to the fact that fecal samples were collected from extensively managed animals which were kept out doors and were less exposed to *Cryptosporidium* oocyst than intensively managed sheep where overcrowding and confinement to small areas favor environmental contamination with oocysts and expose animals to infection. Furthermore, in extensive management system, the oocysts are spread to large surface area resulting in low infection pressure.

The present findings in *Cryptosporidium* infections in cattle (34.2%) and in sheep (19.4%) could be a risk to public health, as *Cryptosporidium* is a zoonotic parasite particularly in immunologically compromised individuals. There was a significant difference in *Cryptosporidium* infection in sheep among animals with different body conditions with high prevalence in animals with good body condition (26%) than medium (9.7%) and poor body condition (4.2%). This finding seems to be a paradox and requires further detailed study.

Table 1. Over all prevalence of *Cryptosporidium* infection in cattle and sheep.

Animal species	Number of animals examined	Number positive (%)	X ²	Odds ratio	95% C.I	P value
Cattle	532	182(34.2)	12.415	2.167	1.4-3.354	0.01*
Sheep	155	30(19.4)	-	0.24	-	-
Total	687	212(30.9)	-	-	-	-

*Significant difference.

Table 2. Univariate logistic regression analysis of the association between cattle *Cryptosporidium* infection and the hypothesized risk factors.

Factors	Number of animals examined	Number positive (%)	X ²	Odds ratio	95% C.I	P-value
Breed	-	-	4.049	-	-	0.132
Cross	12	5(41.7)	-	1.8	0.546-5.937	-
Exotic	344	127(36.9)	-	-	-	-
Local	176	50(28.4)	-	Ref.	-	-
Sex	-	-	0.913	-	-	0.339
Female	408	144(35.3)	-	1.234	0.801-1.902	-
Male	124	38(30.6)	-	Ref.	-	-
Age group	-	-	12.55	-	-	0.000*
Adult	327	93(28.4)	-	Ref.	-	-
Young	205	89(43.4)	-	2.767	0.359-3.747	-
Body condition	-	-	11.31	-	-	-
Good	299	120(40.1)	-	1.49	0.816-2.721	0.003*
Medium	175	44(25.1)	-	-	-	-
Poor	58	18(31)	-	Ref.	-	-
Study site/Farm	-	-	48.273	-	-	0.000*
Adama dairy farm	133	58(43.6)	-	2.033	0.97-4.16	-
Adama Vet clinic	36	5(13.9)	-	3.304	1.498-7.284	-
ARPC	63	35(55.6)	-	2.929	0.384-3.24	-
CVMA-VTH	50	13(26)	-	0.701	0.295-1.669	-
EMDI dairy farm	92	40(43.5)	-	2.044	1.011-4.1123	-
Holota dairy farm	45	4(8.9)	-	0.378	-	-
Holota vet clinic	62	13(21)	-	0.384	-	-
Tasew dairy farm	51	14(27.5)	-	Ref.	-	-
Season	-	-	19.979	-	-	0.000*
Dry	425	165(38.8)	-	3.36	1.931-5.84	-
Rainy	107	17(15.9)	-	Ref.	-	-
Agroecology	-	-	20.276	-	-	0.000*
High land	107	17(15.9)	-	0.285	-	-
Low land	169	63(37.3)	-	0.662	-	-
Mid land	256	102(39.8)	-	Ref.	-	-
Production system	-	-	13.331	-	-	0.000*
Extensive	139	30(21.6)	-	Ref.	-	-
Intensive	393	152(38.7)	-	2.631	0.278-3.686	-
Fecal consistency	-	-	1.562	-	-	0.462
Diarrheic	29	13(44.8)	-	1.638	0.741-3.623	-
Dry	319	108(33.9)	-	-	-	-
Moist	184	61(33.2)	-	-	-	-
Over all	532	182(34.2)	-	-	-	-

*Significant difference; **CVMA**: College of Veterinary medicine and agriculture; **ARPC**: Animal research and production center; **VTH**: Veterinary teaching hospital; **EMDI**: Ethiopian meat and dairy institute.

Table 3. Multivariate logistic regression analysis of the association between cattle *Cryptosporidium* infection and the hypothesized risk factors.

Factors	Number of animals examined	Number positive (%)	X ²	Odds ratio	95% C.I	P-value
Age group	-	-	12.55	-	-	0.000*
Adult	327	93(28.4)	-	Ref.	-	-
Young	205	89(43.4)	-	2.767	0.359-3.747	-
Study sites/Farms	-	-	48.273	-	-	0.000*
Adama dairy farm	133	58(43.6)	-	2.033	0.97-4.16	-
Adama Vet clinic	36	5(13.9)	-	3.304	1.498-7.284	-
ARPC	63	35(55.6)	-	2.929	0.384-3.24	-
CVMA-VTH	50	13(26)	-	0.701	0.295-1.669	-
EMDI dairy farm	92	40(43.5)	-	2.044	1.011-4.1123	-
Holota dairy farm	45	4(8.9)	-	0.378	-	-
Holota vet clinic	62	13(21)	-	0.384	-	-
Tasew dairy farm	51	14(27.5)	-	0.426	-	-
Production system	-	-	13.331	-	-	0.000*
Extensive	139	30(21.6)	-	Ref.	-	-
Intensive	393	152(38.7)	-	2.631	0.278-3.686	-
Over all	532	182(34.2)	-	-	-	-

*Significant difference;

CVMA: College of Veterinary medicine and agriculture; **ARPC**: Animal research and production center; **VTH**: Veterinary teaching hospital; **EMDI**: Ethiopian meat and dairy institute.

as opposed to that of 10 who collected samples mainly from dairy calves and lambs.

Conclusion and Recommendations

The present study indicated that *Cryptosporidium* is widely distributed in cattle and sheep in the study areas. Many adult animals were infected with this parasite, meaning that adult animals could also play a significant role in parasite transmission through environmental contamination with oocysts of the parasite. There was a significant difference in the *Cryptosporidium* infection between age groups of cattle, with higher prevalence in young (43.4%) than adult age group (28.4%). Similarly, there was a significant difference in *Cryptosporidium* infection among study sites with higher prevalence in ARPC dairy farm (55.6%) than the rest of the study sites. There was also considerable difference in *Cryptosporidium* infection between production systems, with higher prevalence in the intensive production system (38.7%) than the extensive system (21.6%). There was also a significant difference in the intensity of infection of *Cryptosporidium* ($P= 0.000$) between the age groups of cattle with more burden of illness in the young age category than the adult age group.

Body condition was found to be the only risk factor associated with *Cryptosporidium* infection in sheep. There was no significant difference observed in *Cryptosporidium* infection between the dry and rainy season; among cattle breed and among the different fecal consistency in both cattle and sheep. The majority of *Cryptosporidium* infections in both cattle and sheep were mild.

As a result, further research is needed to fully understand the economic impact of subclinical infection by *Cryptosporidium* species in cattle and sheep. Detailed study on *Cryptosporidium* involving other domestic animals including pet animals should also be initiated; study on the pathogenic effects/pathology of *Cryptosporidium* should be done; drug sensitivity trails on *Cryptosporidium* in domestic animals should be carried out and, finally study involving molecular method to identify the circulating species of *Cryptosporidium* in cattle and sheep should also be conducted.

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