

Occurrence of *Giardia* in Apparently Healthy Cattle and Sheep in Selected Areas of Central Oromia, Ethiopia

Dinka Ayana^{*}, Bersissa Kumsa and Gebayehu Alkadir

Department of Pathology and Parasitology, College of Veterinary Medicine and Agriculture, Addis Ababa University, Addis Ababa, Ethiopia

Abstract

Background: *Giardia* is a ubiquitous intestinal protozoa causing giardiasis worldwide within the vast majority of domestic/wild mammals and humans. There is very few information on the occurrence *Giardia* in cattle and sheep in Ethiopia. This study was conducted from November, 2022 to August 2022 in three selected areas of central Ethiopia (Holeta, Bishoftu and Adama) to estimate the prevalence of *Giardia* infection in cattle and sheep, identify risk factors associated with the occurrence of the parasite and estimate the infection intensity or burden of the parasite.

Materials and methods: 687 fecal samples were collected and examined by zinc sulfate floatation technique using Lugol's iodine solution to identify cysts of *Giardia*. Pictures of *Giardia* cysts were captured using camera fixed to a microscope. The intensity of *Giardia* infection was estimated semi quantitatively by counting the average number of cysts in 10 randomly selected fields of the microscope. The collected data were analyzed using SPSS version 26 statistical software by employing descriptive statistics such as percentage, *Chi square* test, and logistic regression and Mann-Whitney test of non-parametric values.

Results: Out of 687 fecal samples examined by coprology, 170 (24.7%) of cattle and sheep were infected with *Giardia*. The specific prevalence of *Giardia* in cattle and sheep was 27.4% and 15.5% respectively.

Conclusions: Breed, body condition, study site/farm, production system and fecal consistency were found to be risk factors associated with the occurrence of *Giardia* infection in cattle while only agro ecology was found to be associated with the occurrence of *Giardia* infection in sheep. Majority of *Giardia* infections in both cattle and sheep were found to be mild. The study indicates that *Giardia* infections are widely distributed in cattle and sheep in the study areas and require proper control and prevention measures.

Keywords: Adama • Bishoftu • *Giardia* • Holeta • Species • Prevalence

Introduction

Giardiasis is, a protozoan disease that is widely distributed and traditionally considered as an epidemic and zoonotic disease between human and animals (farm animals, dogs, cats, birds and rodents) affecting all age groups [1]. *Giardia* is a ubiquitous, intestinal protozoa causing giardiasis worldwide within the vast majority of domestic/wild mammals and humans [2]. It is common in cattle and causes gastrointestinal problems including diarrhea [3]. Even though this infection is often subclinical or even asymptomatic, it causes acute or chronic diarrhea, reduced weight gain and ill thrift [4]. There are six known *Giardia* species; but, only *Giardia duodenalis* (syn. *Giardia intestinalis*, *Giardia lamblia*) has been found to infect humans and other mammals [5]. In its life cycle, *Giardia* has two life phases,

trophozoite and cyst. The trophozoite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission. *Giardia* infection occurs through ingestion of contaminated water and food by *Giardia* cysts or by the fecal oral route (through poor hygiene practices). The cyst can survive for weeks to months in warm water [6].

A high prevalence of Giardiasis has been reported worldwide in dairy and beef cattle [7, 8]. Due to the great number of cattle and the associated large feces output that contributes to the high prevalence of *Giardia* infection, bovine giardiasis is of great concern. Molecular studies have confirmed that the prevalence of giardiasis in cattle ranges from 2.2 to 50.7% worldwide [9]. This high prevalence of giardiasis in cattle plays important role in causing human giardiasis. Some studies have revealed that close

***Address for Correspondence:** Dinka Ayana, Department of Pathology and Parasitology, College of Veterinary Medicine and Agriculture, Addis Ababa University, Addis Ababa, Ethiopia, Tel: 251953000000; E-mail: dinka.ayana@aau.edu.et

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contact of humans with farm animals is associated with high prevalence of human giardiasis.

In tropical region, giardiasis is one of the causes of serious diarrhea in calves [10-12]. It has emerged as important parasitic disease of dairy cattle because of its proven pathogenicity, economic losses and the potential public health significance of zoonotic transmission [13].

In cattle, direct transmission through the contamination of surroundings by the feces of infected animals seems to be the principal mode of infection. Farm animals play significant role in contributing parasite cysts in large proportion because of their high abundance on farms and can serve as the causal agents of human giardiasis. Transmission to humans may occur through either direct contact in the case of farmers, veterinarians, and petting zoos, or through indirect routes such as contaminated surface water or food [14].

Sources of contamination of water and food might be diverse, but a particularly important, though varying, role is played by different host groups that act as reservoirs of infection. So, control of giardiasis in cattle has become important, not only to reduce the risk of disease in cattle, but also to reduce the risk of infection in humans [15].

The prevalence and distribution of bovine and sheep giardiasis have been reported in various countries globally; however, it is excluded from reports on animal morbidity and mortality in Ethiopia. There have been only two studies conducted on *Giardia* infection in cattle in Ethiopia; one by Wegayehu T who reported an overall prevalence of 2.3% in North Shewa and another report by Yimer Muktar with 22.9% overall prevalence in purposively studied diarrheic calves in Muke Turi, Debre Tsige and Fiche towns of North Shewa, Ethiopia [16,17]. As a result, there is scarcity of information on the prevalence, species diversity and associated risk factors of *Giardia* infection in ruminants and its public health significance in Ethiopia. Furthermore, the role of *Giardia* infection in ruminants as a cause of disease and production losses has been neglected in Ethiopia. Therefore, this study was initiated to estimate the prevalence of *Giardia* infection in cattle and sheep, to estimate infection intensity/burden of the parasite and identify potential risk factors associated with the occurrence of the parasite.

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).

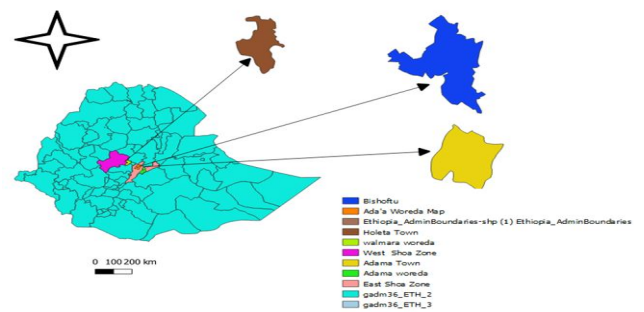


Figure 1. Map showing the study areas.

Bishoftu town is located at 9°N latitude and 40°E longitudes, 47 km South-East of Addis Ababa, at an elevation of 1850 meters 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. 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.

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 rainfall of 834-1300 mm and the annual temperature of 11°C-22°C. Rainy season occurs with bimodal distribution 70% of which occurs during the main rainy season (June to September) and 30% during the short rainy season (February to April) and relative humidity of 50.4%. 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 labour 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.

Adama is a town 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 13.9°C-27.7°C, respectively. The town is one of the most populous from the regional state and is located at an important multidirectional trade route. The population 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 [18].

Cattle and sheep managed under both intensive (Dairy farms) and extensive productions systems were considered for the study. 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 years; adult more than two years; for sheep young refers to less than one year and adult is more than one year) according to Heidari H [19]. The body condition of cattle and sheep was categorized as poor, medium and good respectively as per the guide lines by Klopčič M [20].

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. While 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 Thrusfield M with 95% confidence interval and 5% absolute precision.

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

Where, P_{exp} = Expected prevalence; d = Absolute precision; N = Sample size.

Considering 15.8% expected prevalence of intestinal protozoal infections in cattle 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 $204 \times 2 = 408$. However, we increased the sample size to 687 samples.

Animal species	Number of animals examined	Number of positive animals (%)	χ^2	Odds ratio	95% C.I.	P-value
Bovine	532	146(27.4)	9.219	2.065	1.284-3.320	0.003*
Ovine	155	24 (15.5)		Ref.		
Total	687	170 (24.7)				

Note: * : Significant difference

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

Giardia infection in cattle

From a total of 532 fecal samples examined by coproscopy, 146 cattle (27.4%) were found to be infected by *Giardia* parasite. Univariate logistic regression analysis of the association between *Giardia* infection and the various factors showed that seven risk factors (breed, body condition, and study site/farm, season of the

Sample collection and laboratory analysis

Coprological examination: About 10 gram 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/screw cup, preserved in 10% formalin with the ratio of 1 gram of feces to 3 ml of formalin to prevent the *Giardia* cyst from desiccation, and transported in ice box to the parasitology laboratory of Addis Ababa university, college of veterinary medicine for laboratory analysis. Zinc sulphate flotation (SG of 1.18) technique with lugol's iodine staining or without staining was used to identify *Giardia* cysts and trophozoites. An animal was considered *Giardia* positive if at least one *Giardia* cyst was detected with the correct morphology (*i.e.* optical properties, internal structure, size and shape).

Estimation of intensity of *Giardia* infection: Intensity of infection of *Giardia* was estimated in such a way those *Giardia* cysts positive 10 random fields were counted and the estimated cyst count/gram was estimated by multiplying by the number of fields on the cover slip.

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 protozoa parasites at a desired precision level of 5% and confidence interval of 95%. Significant difference was considered when $P < 0.05$.

Results

Over all *giardia* infections in cattle and sheep

Out of 687 animals examined by coprology, 170(24.7%) of the animals were infected with *Giardia*. There was a significant different ($P=0.003$) in *Giardia* infection between the species of the study animals with higher prevalence in cattle (27.4%) than sheep (15.5%) (Table 1).

year, agro ecology, production system and fecal consistency) were associated with the occurrence of cattle *Giardia* infection (Table 2). Nevertheless, multivariate logistic regression analysis revealed that breed, body condition, study site/farm, production system and fecal consistency were identified as potential risk factors associated with cattle *Giardia* infection (Table 3).

Variables	Category	Number of animals examined	Number of positive animals (%)	χ^2	Odds ratio	95% CI	P value
Breed	Cross	12	3 (75)	4.702	1.595	1.042-2.443	0.032*
	Exotic	344	105 (30.5)		1.211	0.312-4.693	
	Local	176	38 (21.6)		Ref.		
Sex	Female	408	110 (27)	0.205	0.409		0.651
	Male	124	36 (29)		Ref.		
Age group	Adult	327	95 (29.1)	1.102	1.236	0.832-1.838	0.294
	Young	205	51 (24.9)		Ref.		
Body condition	Good	299	100 (38.4)	12.59	2.412	1.71-4.967	0.002*
	Medium	175	36 (20.6)		1.243	0.574-2.695	
	Poor	58	10 (17.2)		Ref.		
Study site	Adama dairy farm	133	14 (10.5)	63.361			0.000*
	Adama vet clinic	36	6 (16.7)				
	ARPC	63	18 (28.6)				
	EMDI dairy farm	92	47 (51.5)		1.086	0.548-2.154	
	Holota dairy farm	45	11 (24.4)				
	Holota vet clinic	62	10 (16.1)				
	Tasew dairy farm	51	25 (49)				
	CVMA-VTH	50	15 (30)				
Season	Dry	425	125 (29.4)	4.111	1.708	1.014-2.872	0.044*
	Rainy	107	21 (19.6)				
Agroecology	High land	107	21 (19.6)	5.16	0.351	0.205-0.601	0.000*
	Low land	169	20 (11.8)		Ref.		
	Mid land	256	105 (41)				
Production system	Extensive	139	29 (20.9)	4.092	Ref.		0.043*
	Intensive	393	117 (29.8)		0.622	0.392-0.988	
Fecal consistency	Diarrheic	29	7 (24.1)	8.789			0.013*
	Dry	319	74 (23.2)		Ref.		
	Moist	184	65 (35.3)		0.583	0.371-0.824	
Over all		532	146 (27.4)				

Note: *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 2. Univariate logistic regression analysis of the association between cattle *Giardia* infection and the assumed risk factors.

Variables	Category	Number of animals examined	Number of positive animals (%)	χ^2	Odds ratio	95% CI	P value
Breed	Cross	12	3 (75)	4.702	1.595	1.042-2.443	0.032*
	Exotic	344	105 (30.5)		1.211	0.312-4.693	

	Local	176	38 (21.6)		Ref.		
Body condition	Good	299	100 (38.4)	12.59	2.412	1.71-4.967	0.002*
	Medium	175	36 (20.6)		1.243	0.574-2.695	
	Poor	58	10 (17.2)		Ref.		
Study site	Adama dairy farm	133	14 (10.5)	63.361			0.000*
	Adama vet clinic	36	6 (16.7)				
	ARPC	63	18 (28.6)				
	EMDI dairy farm	92	47 (51.5)		1.086	0.548-2.154	
	Holota dairy farm	45	11 (24.4)				
	Holota vet clinic	62	10 (16.1)				
	Tasew Dairy farm	51	25 (49)				
	CVMA-VTH	50	15 (30)				
Production system	Extensive	139	29 (20.9)	4.092	Ref.		0.043*
	Intensive	393	117 (29.8)		0.622	0.392-0.988	
Fecal consistency	Diarrheic	29	7 (24.1)	8.789			0.013*
	Dry	319	74 (23.2)				
	Moist	184	65 (35.3)		0.583	0.371-0.824	
Over all		532	146 (27.4)				

Note: *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 *Giardia* infection and the assumed risk factors.

Giardia infection in sheep

Out of 155 fecal samples examined by coprology, 24 (15.5%) sheep were found to be infected by *Giardia* parasite. Univariate

logistic regression analysis of the association between *Giardia* infection and the various factors indicated that only agro ecology was associated with the occurrence of sheep *Giardia* infection (Table 4).

Variables	Category	Number of animals examined	Number of positive animals (%)	χ^2	Odds ratio	95% CI	P value
Sex	Female	93	17 (18.3)	1.389	1.758	0.682-4.527	0.239
	Male	62	7 (11.3)		Ref.		
Age group	Adult	112	17 (15.2)	0.029	Ref.	0.362-2.404	0.865
	Young	43	7 (16.3)		0.194		
Body condition	Good	100	19 (19)	2.681	2.58	0.558-11.932	0.262
	Medium	31	3 (9.7)		1.179		
	Poor	24	2 (8.3)		Ref.		
Season	Dry	119	19 (16)	0.091	1.178	0.406-3.415	0.763
	Rainy	36	5 (13.9)		Ref.		
Agro ecology	High land	36	5 (13.9)	5.11	0.53	0.173-1.62	0.033*
	Low land	59	5 (8.5)		Ref.		
	Mid land	60	14 (23.3)		2.304	0.102-2.908	

Fecal consistency	Diarrheic	6	1 (16.7)	0.075	0.808	0.163-4.005	0.963
	Dry	138	21 (15.2)				
	Moist	11	2 (18.2)				
Study site	Adama vet clinic	59	5 (8.5)	5.11	1.887	0.617-5.772	0.09
	CVMA-VTH	60	14 (23.3)				
	Holota Vet clinic	36	5 (13.9)				
Over all		155	24 (15.5)				

Note: *Significant difference, CVMA: College of Veterinary Medicine and Agriculture; VTH: Veterinary Teaching Hospital

Table 4. Univariate logistic regression analysis of the association between sheep *Giardia* infection and the assumed risk factors.

Intensity of *Giardia* infection

The average number of *Giardia* cysts was counted to assess the intensity/burden of infection in both study animals. Cysts of *Giardia* are oval shaped, thin walled cyst that is 10 to 20 µm in length, 7-10 µm in width and 0.3-0.5 µm thickness in morphology (Figure 2). The maximum and minimum mean count of *Giardia* cysts in cattle were 50 and zero respectively (Figure 3). In sheep, the maximum and minimum number of *Giardia* cysts was 15 (0.6%) and zero (85.2%) respectively (Figure 4).

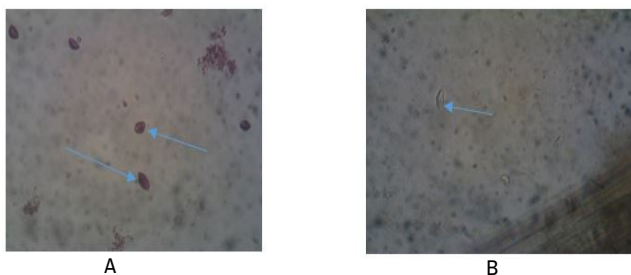


Figure 2. A) *Giardia* cysts from Bovine (Lugol's iodine stain) B) *Giardia* cyst (Unstained) cysts of *Giardia* seen under the microscope by zinc sulphate flotation.

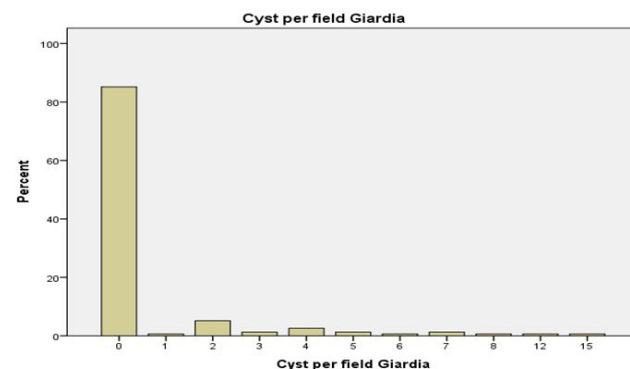


Figure 4. Average number of *Giardia* cysts in sheep feces.

Age related intensity of infections of intestinal protozoa

Mann-Whitney test analysis indicated that there was no significant difference in the intensity of infections of *Giardia* in both cattle and sheep between the two age categories.

Discussion

In the present study, the prevalence of *Giardia* in cattle and sheep was 27.4% and 15.5% respectively. The present report in the prevalence of cattle *Giardia* is greater than the reports of Bruce R Hoar, et al. (19.1%) from central and western USA; Iburg T, et al., (7.6%) from Denmark; Getrude Shepelo Peter, et al., (0%) from Kenya; Khin Sandar Lwin. (0%) from Thailand; (2.3%) from Girar Jarso and Dera, Noth Shewa, Ethiopia; (10.6%) from Gamo Gofa, Ethiopia; Wegayehu T (9.6%) from central Ethiopia; Roberta dos Santos Toledo (7.6%) from Parana, Brazil and Maria Vitale (9.7%) from south Ethiopia. However, the present prevalence of cattle *Giardia* was lower than that reported by Al-Saad RK (31.6%) in cattle from Iraq and that of Mahato MK (44.79%) in dairy cattle of Chitwan, Nepal.

The current sheep *Giardia* prevalence report is relatively similar to the reports of Jun-Jie Peng, 2020 (14.5%) from China; Muhammad Imran, 2013(16%) from Pakistan; Rongchang Yang, 2009 (11.1%) from Australia.

Our prevalence in *Giardia* infection in sheep was higher than the reports of Weizhe Zhang, (5.6%) from China; Junqiang Li (6.65%) from China; Rongchang Yan (6.3%) from Western Australia. However, our finding was lower than that reported by

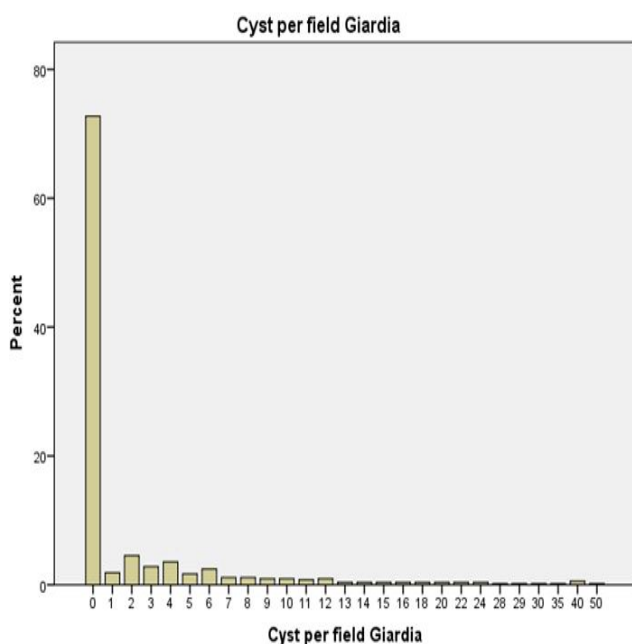


Figure 3. Average number of *Giardia* cysts in cattle feces.

Nikolaos Tzanidakis, (37.3%) from Greece. The variation in the prevalence reports of *Giardia* infections from different areas could be due to differences in agro climates, season of fecal samplings, husbandry practices and the laboratory techniques used for the studies and the intermittent nature of oocyst shedding by the parasite.

There was a significant difference in cattle *Giardia* infection among breeds of the cattle with higher prevalence in cross bred (75%) followed by exotic breed (30.5%) and local breed (21.6%). This could be related to high buildup of *Giardia* cysts in dairy farms which expose animals to infection compared to those managed under extensive management system (local breeds). This could also be associated with the immune status of the animals the fact that local animals are relatively resistant to infections compared to pure and cross breeds of cattle. There is also a significant difference in *Giardia* infection between animals among the body conditions with higher prevalence in animals with good body condition (38.4%) than medium (20.6%) and poor body condition (17.2%).

Similarly, there was a significant difference in *Giardia* infection among study sites/dairy farms. High prevalence (51.5%) and least prevalence (10.5%) were recorded in EMDI dairy farm and Adama dairy farm respectively. This variation could be associated with hygienic status and overall management practices of the dairy farms. Dairy farms with poor hygiene usually experience high risk of parasitic infections.

There was also a significant difference in cattle *Giardia* infection between production systems. Higher prevalence (29.8%) was reported in intensively managed animals compared to those from extensive production system (20.9%). This could be associated to high buildup of *Giardia* cysts in dairy farms resulting in more exposure of animals to infections. There could also be overcrowding in dairy farms which could lead to stress resulting in high susceptibility of the host to *Giardia* infection. There was also significant association in *Giardia* infection between fecal consistency with more occurrence in moist feces than diarrheic and dry feces.

Statistically, significant difference was observed in sheep *Giardia* infection among different agro ecologies with high prevalence in mid land which is Bishoftu (23.3%) than high land/Holeta (13.8%) and low land (Adama) (8.5%). This could be due to the favorable climatic conditions of mid land environment for the parasite development and transmission as compared to low land and high land agro ecologies.

The maximum and minimum mean count of *Giardia* cysts per gram of feces in cattle was 50 and zero respectively. The corresponding average number of cysts per gram of feces in sheep was 15 (0.6%) and zero (85.2%) respectively. There was no significant difference observed in the intensity *Giardia* infections between the age groups of cattle and sheep. This finding disagrees with the report of Miguella P, et al., who recorded high cysts shedding by young animals compared to adult ones indicating that the intensity of shedding *Giardia* cysts decreased significantly with the age of the animal.

The present finding in infection intensity of *Giardia* is lower than that of Jose Antonio Castro-Hermida, et al., who reported minimum and maximum of 15 and 3,042 cysts per gram of feces respectively in calves and 16-3010 CPG (cysts per gram of feces) from sheep in Spain. Our finding was also lower than that of Iburg T, who recorded 200 cysts per gram of feces in calves. The reason for such low excretion is unclear but possible explanation could be due to the fact

that in our present study, majority of the study animals were from adult age group which usually shed fewer number of *Giardia* cysts compared to the young age group. It must also be noted that fecal samples were examined on one occasion and it is possible that some animals were infected but were not shedding cysts and that others were shedding low numbers at the time of sampling and examination. Clearly, further work is necessary to study the dynamics of infection over a long period of time. Although the number of cysts excreted from cattle and sheep in the present study seem low, they represent large number of cysts per kilogram of feces and these numbers may be quite significant from epidemiological point of view i.e. environmental contamination with cysts of *Giardia*.

Conclusion and Recommendation

The present study indicated that *Giardia* was widely distributed in cattle and sheep in the study areas. Breed, body condition, study site/farm, production system and fecal consistency were the risk factors associated with the occurrence of *Giardia* infection in cattle while agro ecology was found to be the only risk factor associated with the occurrence of *Giardia* infection in sheep. The majority of *Giardia* infections in both cattle and sheep were found to be mild. Significant number of adult animals was infected with *Giardia*. This finding implies that adult animals can serve as reservoirs of the infections in livestock and play a great role in the parasite transmission through environmental contamination with cysts of *Giardia*. Study involving molecular method to identify the circulating species/assemblages of *Giardia* in cattle and sheep should be conducted.

Ethics Approval and Consent to Participate

All the studies were performed in accordance with the animal research and ethical guidelines and regulations of the college of veterinary medicine and agriculture of Addis Ababa university. All the study methodologies were reported in accordance with ARRIVE guidelines. The study methodologies were reviewed and approved by the animal research ethical review committee of the college of veterinary medicine and agriculture of Addis Ababa university (Ref no. VM/ERC/28/03/14/2022).

Consent for Publication

Not applicable

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors have no competing interest.

Funding

No funding

Authors' Contributions

Study concept and design: Dinka Ayana, Bersissa Kumsa

Data collection: Dinka Ayana and Gebayehu Alkadir

Data analysis, interpretation and draft manuscript preparation: Dinka Ayana

All authors reviewed the results and approved the final version of the manuscript.

Ethical Considerations

The ethical clearance was obtained from the ethical review committee of the college of veterinary medicine and agriculture of Addis Ababa university (Ref no. VM/ERC/28/03/14/2022).

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