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The Prevalence of *Giardia* Infection and Associated Risk Factors in Selected Dairy Farms in Akaki Kality Subcity, Addis Ababa, Ethiopia

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Abstract: A cross sectional epidemiological study design was employed to estimate the prevalence of Giardia infection and to identify the associated risk factors in intensive and semi- intensive dairy farms in Akaki kality, Addis Ababa. Accordingly, 384 cattle were selected through multistage random sampling and fecal samples were collected from each sampling unit. Formalin ether concentration technique was used to identify *Giardia* from the fecal samples. An overall prevalence of bovine *Giardia* infection was found to be 3.1% (12/384). The risk of *Giardia* infection was found significantly higher in poor body condition cattle than in cattle with good body condition (OR=11.05, CI= 1.25-97.43, p= 0.03). The prevalence of the infection was more than four folds in pre weaned calves than in greater than 6 month of age calves (OR=4.53, CI=1.06-19.36, p= 0.042). Similarly, in 2 to 6 month of age calves, the risk of *Giardia* infection was more than five fold higher compared to cattle of greater than 6 months of age (OR=5.5, CI=1.20-25.28, p= 0.028). Despite higher prevalence of *Giardia* infection in semi intensive management system (5.40%) than in intensive management system (1.69%), there was no statistically significant difference (p=0.054). There was statistically significant difference in the prevalence of giardia infection in diarrheic cattle than in non-diarrheic cattle (OR=7.66, CI=2.25-26.11). In conclusion, even though lower overall prevalence was recorded, findings from this study indicated that Giardiasis is one of the obstacles of dairy production in Akaki kality subcity of Addis Ababa, Ethiopia.

Key words: Akaki Kality · Giardia · Prevalence

INTRODUCTION

Despite the large livestock population in Ethiopia (49.3 million heads of cattle) [1], the economic benefits remain marginal due to prevailing diseases, poor nutrition, poor animal production systems, reproductive inefficiency, management constraints and general lack of veterinary care [2]. Among the diseases, parasitic diseases are considered as major obstacles in curtailing health and lowering productivity of animals [3]. One of these parasitic diseases is *Giardiasis,* 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 [4].

Giardia is a ubiquitous, intestinal protozoa causing *Giardiasis* worldwide within the vast majority of domestic/wild mammals and humans [5]. It is common in cattle and causes gastrointestinal problems including diarrhea [6]. Even though this infection is often subclinical or even asymptomatic, it causes acute or chronic diarrhea, reduced weight gain and ill thrift [6, 7]. There are six known *Giardia* species; but, only *Giardia duodenalis* (syn. *Giardia intestinalis, Giardia lamblia*) has been found to infect humans and mammals [8]. In its life cycle, it has two life phases, trophpzoite and cyst. *Giardia* infection can occur through ingestion of dormant cysts in contaminated water, food, or by the fecal-oral route (through poor hygiene practices). The *Giardia* cyst can survive for weeks to months in warm water [9]. The trophozoite attaches to the epithelium by a ventral adhesive disc and reproduces via binary fission [10].

A high prevalence of *Giardiasis* has been reported worldwide in dairy and beef cattle [6, 11]. Due to the great number of cattle and the associated large feces output that contributes to the high prevalence of *Giardia*

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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 [12]. This high prevalence of Giardiasis in cattle plays important role in causing human Giardiasis [10]. Some studies have revealed that close contact of humans with farm animals is associated with the high prevalence of human Giardiasis [13].

In tropical region, Giardiasis is one of the causes of serious diarrhea in calves [14, 15, 16]. 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 [6, 15].

Transmission of *Giardia duodenalis* infection occurs via cysts, which are excreted in the feces of infected humans and animals [5]. 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 [5, 16, 17]. 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 foods [18].

Epidemiological surveys indicated that the most important sources of human infection are contaminated drinking and recreational water, food, household animals and infected people. 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. Farm animals are believed to play the most significant role in this context, contributing parasite cysts in large proportion because of their high abundance on farms [19]. 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 [20].

The prevalence and importance of bovine Giardiasis have been documented in various countries of the world; however, it is excluded from reports on animal morbidity and mortality in Ethiopia. To the author's knowledge, there have been only two studies conducted about *Giardia* infection in cattle in Ethiopia one by Wegayehu *et al.* [21] who reported an overall prevalence of 2.3% in North Shewa and another report by Yimer Muktar [22] with 22.9% overall prevalence in purposively studied diarrheic calves in Muke Turi, Debre Stige and Fiche towns of North Shewa, Ethiopia. As a result, there is scarcity of information on the prevalence and associated risk factors of *Giardia* infection in bovine in Ethiopia. Furthermore, the role of *Giardia* infection in cattle as a cause of disease and production losses has been neglected. Therefore, taking into account the role of the parasite as one of the most significant causes of economic losses and the scarcity of information in the country Ethiopia, this study was designed:

- To estimate the prevalence of *Giardia* infection
- To identify the associated risk factors with giardia infection

MATERIALS AND METHODS

The Study Area: The study was conducted in Akaki Kality subcity of Addis Ababa Ethiopia. Akaki kality is one of the 11 subcities of Addis Ababa, the capital city of Ethiopia, located at longitude 38° 44' 24? E and latitude 9° 1' 48? N. Its altitude is 2500 meters above sea level (masl). The annual rainfall is 800–1100 mm and the mean annual maximum and minimum temperature is about 21°C and 27°C, respectively [23]. There were a total of 669 small, medium and large/commercial dairy farms some with intensive and others with semi-intensive management systems in the study area. In the intensive management system, animals are always kept in doors with zero grazing while in the semi-intensive management system, they are kept partly in doors and partly outdoor where they are allowed to graze.

Study Population: Local and crossbreed dairy cattle found within Akaki kality subcity was the study population. The sampling unit of the study was the individual animal. The status of the disease was determined in relation to categories of age of pre weaned (less than 2 months old), 2 months to 6 months old and greater than 6 months old), breeds (local and crossbred), farm management system (intensive and semi intensive), fecal consistency (diarrheic and non-diarrheic) and sex (male and female). The age of the study animals was determined by dental eruption formula which involves counting the number of permanent incisors [24] or based on the breeding records kept by owners. Study animals were also grouped as good, medium and poor based on their body condition score according to the standard set by De la Rua-Domenech [24].

Study Design and Sample Size Determination: A cross-sectional epidemiological study design was conducted from December 2021 to April 2022 to estimate the

prevalence of *Giardia* infection in cattle in Akaki Kality subcity of Addis Ababa. To estimate the sample size, an expected prevalence of 50% was taken into consideration since there was no research work on the *Giardia* infection in cattle in the study area. The desired sample size for the study was then calculated using the formula given by Thrusfield [25] with 95% confidence interval and 5% absolute precision.

So,

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

where, Pexp = expected prevalence; d= absolute precision; N =sample size. Based on this formula, the estimated sample size was 384 animals. Accordingly, 384 animals were sampled.

Sampling Technique and Sample Collection: Dairy farms were selected using multi-stage sampling in which 6 districts were first randomly sampled from the 11 districts in the sub city. 30 dairy farms were again selected randomly from the selected districts. A third random sampling was conducted in including 384 total individual animals of any age, sex and breed categories.

About 30g fresh fecal samples were collected from each study animal either 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 the *Giardia* cyst from desiccation and transported in ice box to the parasitology laboratory of Addis Ababa University College of Veterinary Medicine and Agriculture on the same day of collection where they were preserved at refrigeration temperature until processing using the following laboratory technique within 48 hours of arrival.

Formalin Ether Concentration: For each sample collected, about 7 ml of 10% formalin was added to approximately 1 gram of feces and mixed using an applicator stick. The fecal sample was sieved with cotton gauze and transferred to 15 ml centrifuge tube. After adding 3 ml of diethyl ether to the mixture and hand shaken, the content was centrifuged at 2000 rpm for 3 minutes. The supernatant was poured away and a drop of sediment was transferred to slide. Using pipette, a single drop of Lugol's iodine solution was added to the sediment on the slide and covered with coverslip. Finally, the entire area under the cover slip was systematically examined using 10X and 40X objective lenses of compound microscope to observe the *Giardia* cyst [26]. Presence of one or more oval shaped cysts approximately 8-15 µm long and 7-10 ìm wide with 4 nuclei under the microscope were taken as *Giardia* positive [27].

Data Analysis: Data files were manually entered into Microsoft Excel 2010 spreadsheet and analyzed with STATA Version 12. The descriptive statistics, percentage, was used to determine the level of overall prevalence and the prevalence in relation to different risk factors of the infection. Odds ratio (OR) and its associated confidence interval (CI) with 95% confidence level was calculated using univariate logistic regression analysis to determine the degree of association of the disease with each risk factors. The statistical association between risk factors and the disease was evaluated using p-value and the difference in the prevalence of the disease was regarded statistically significant when p-value was less than 0.05.

RESULTS

Overall Prevalence of Giardia Infection: A total of 384 fecal samples were collected from 384 cattle. Two cattle breeds were included: 248 (64.6%) crossbreed cattle and 136 (35.4%) local breed cattle. Of the total sampled cattle, 265 (69.0%) were females and the remaining 119 (31.0%) were males. The study animals were grouped in to three age groups in which 90 (23.4%), 60 (15.6%) and 234 (60.9%) of cattle were less than two months, 2 to 6 months and above 6 month of age respectively. 61.5% and 38.5% of the sampled animals were kept in intensive and semi intensive management systems respectively. Among the total number of cattle fecal samples examined using formalin ether concentration technique, 12 of them were positive for *Giardia duodenalis* indicating an overall prevalence 3.1% (12/384) (Table 1).

Prevalence of Giardia Infection in Relation to Breed, Sex and Farm Management System: In a univariat analysis, the prevalence of *Giardiasis* was higher in crossbreeds when compared to local breeds. Out of 248 fecal samples of the crossbreeds, 9 were found to be positive (3.6%; 95% CI 1.3%-6.0%) whereas, in the local breed cattle, 3 out of 136 (2.2%; 95% CI 0.3%–4.7%) were positive for the disease. These data revealed that there was no statistically significant association between the cattle breeds and prevalence of *Giardiasis* (p>0.05) (Table 1).

Table 1: Univariate analysis for prevalence of *Giardiasis* relative to cattle breed, sex and farm management system

Variables	Number examined	Number positive	Prevalence (%) (95% CI)	OR (95% CI)	P-value
Breed					
Local	136	3	2.2 (0.3%-4.7%)	Reference	Reference
Crossbreed	248	9	3.6 (1.3%-6.0%)	1.67 (0.44-6.27)	0.443
Sex					
Female	265	8	3.02 (0.9%-5.1%)	Reference	Reference
Male	119	4	3.36 (0.1%-6.6%)	1.12 (0.33-3.79)	0.858
Management system					
Intensive	236	4	1.69 (0.0004-0.0335)	Reference	Reference
Semi-intensive	148	8	5.41 (0.017-0.091)	3.31 (0.98-11.21)	0.054

Table 2: Univariate analysis for prevalence of *Giardiasis* in relation to body condition, age group and fecal consistency

Variables	Number examined	Number positive	Prevalence (%) (95% CI)	OR (95% CI)	P-value
Body condition					
Good	96	1	1.04 (1.0%-3.1%)	Reference	Reference
Medium	240	6	2.50 (0.51%-4.49%)	2.44 (0.29-20.51)	0.413
Poor	48	5	10.42 (1.66%-19.18)	11.05 (1.25-97.43)	0.031
Age					
>6 months	234	3	1.28 (0.17%-2.73%)	Reference	Reference
2-6 months	60	4	6.67 (0.28%-13.05%)	5.5 (1.20-25.28)	0.028
<2 months	90	5	5.56 (0.78%-10.33%)	4.53 (1.06-19.36)	0.042
Fecal consistency	299	4	1.34 (0.0003-0.0265)	Reference	Reference
Non-diarrheic Diarrheic	85	8	9.41 (0.031-0157)	7.66 (2.25-26.11)	0.001

The analysis also showed a 3.6% (95% CI 0.1%-6.6%) prevalence of *Giardiasis* in males which was a little bit higher than the 3.02% (95% CI 0.9%-5.1%) prevalence in females. However, there was no statistically significant association between sex and *Giardia* infection (P > 0.05) (Table 1). The overall prevalence according to management system was determined and it was 5.41% (95% CI 1.7%-9.1%) in semi intensive and 1.69% (95% CI 0.04%-3.35%) in intensive dairy cattle. There was no statistically significant difference (P>0.05) between the two management systems (Table 1).

Prevalence of Giardia Infection in Relative to Age, Body Condition and Fecal Consistency: The analysis showed that the prevalence was higher in calves of 2 to 6 months old 6.67% (95% CI 0.28%-13.05%)) than those calves pre weaned (less than 2 months old) 5.56% (95% CI 0.78%-10.33%) and above 6 month of age 1.28 (95% CI= 0.17%-2.73%). There was statistically significant difference between the two age groups (p-value<0.05) (Table 2). Cattle belonging to poor body condition showed significantly higher prevalence 10.42% (95% CI =1.66%-19.18%) than those belonging to Medium 2.5% (95% CI= 0.51%-4.49%) and good 1.04% (95% CI= 1.0%-3.1%) body condition in the study area (Table 2). The association between body condition and *Giardiasis* infection was statistically significant (P<0.05).

Gross examination of fecal samples showed that 22.1% of the total (85/384) was diarrheic out of which 9.41% (95% CI 3.1%-15.7%) was *Giardia* positive through the formalin ether concentration procedure. The remaining 77.9% (299/384) of the sample was non diarrheic with 1.34% (95% CI 0.03%-2.65) positive for the test. The result indicated that there was statistically significant association (P<0.05) between infection with *Giardiasis* and fecal consistency (Table 2).

DISCUSSION

This study was the first to report the prevalence of *Giardia* infection in cattle in the study area. The overall prevalence of bovine *Giardia* infection in this study (3.1%, 12/384) was relatively similar to the report by Wegayehu *et al.* [21] (2.3%) in North Shewa Zone, Ethiopia and that of Degerli *et al*. [28] who reported (3.7%) prevalence in in Turkey. The present finding is however lower than the prevalence reports (22.5%, 14%, 7.2%, 23% and 34.5%)n from Myanmar, Canada, Germany, Sweden and Argentina respectively by Bawm *et al*. [29], Bjorkman *et al*. [30], Budu–Amoako *et al*. [31], Gillhuber *et al*. [32] and Tiranti *et al*. [33] but higher than a zero prevalence reported by Randhawa *et al*. [34] in Kenya. The overall prevalence 22.9% reported byYimer Muktar [22] in purposively selected diarrheic calves in

Muke Turi, Debre Stige and Fiche towns of North Shewa, Ethiopia was also higher than the current finding. There are many factors that complicate the detection rate of infection including; the sensitivity and specificity of diagnostic techniques, the size of specimens and the age and health status of the infected host. Compared to molecular methods, microscopy methods have yielded false negatives for fecal specimens with a low intensity of *G. duodenalis* cysts [35]. Some authors also suggest that the prevalence values might be underestimated, since *Giardia* cyst excretion is intermittent [36]. So, in the present study, the low prevalence observed might be mainly related to the large percentage of mature animals in the sample population as well as the low sensitivity of morphology method of *Giardia* cyst detection used as compared to most of reports conducted through molecular detection techniques.

The highest prevalence was observed in calves of 2 to 6 months old (6.67%) followed by preweaned calves < 2 months old (5.56%) and cattle > 6 months old (1.28%). This trend contradicted to the finding of Budu Amoako (2012) who reported a highest prevalence in pre weaned calves < 2 months old (58%) with the intensity of infection decreasing through post weaned calves 2–6 months of age (40%) and animals > 6 months old (25%). The present result also indicated that age was significantly associated with *Giardia* infection in which calves of 2 to 6 months old were 5.5 times more likely at risk of infection than above 6 months old calves (OR=5.5, CI=1.20–25.28, P=0.028). The infection was also found 4.53 times more prevalent in preweaned calves than in above 6 months old cattle (OR=4.53, CI=1.06–19.36, P=0.042) which was nearly similar with the finding by Kebede *et al.* [3] in Myanmar who reported a prevalence of 2-fold *Giardia* infection in calves younger than 6 months old compared to those older than six months of age. This could be due to strong immune response of adults to diseases as compared to young animals. Calves are born immunologically naïve (immature immunity) [37] and their immunity matures with time in which calves beyond 6 months of age are able to mount a higher primary immune response [38]. Therefore, the development of some immunity could have partially led to the lower prevalence of the disease in those cattle above 6 month of age as compared to those less than 6 month of age. The higher prevalence of *Giardia* infection found in 2 to 6 months old calves (6.67%) than preweaned calves (5.56%) may be related to increasing susceptibility of 2 to 6 months old calve to diseases due to weaning stress. The young calves (preweaned calves) can also be protected from *Giardia* infection by consumption of colostrums compared to weaned calves [39].

A strong association of *Giardia* infection in relation to the body condition of study animals has been demonstrated in this study. Consequently, cattle belonging to poor body condition showed significantly higher prevalence than cattle belonging to Medium and good body condition. Quantitatively, the prevalence of the disease in cattle of Medium body condition was 2.44 times (OR=2.44, 95% CI=0.29-20.51) higher than its prevalence in good body condition cattle. Similarly, the infection was 11 times more prevalent in poor body condition animals than good body condition animals (11.05, 95% CI 1.25-97.43). The reason may be related to the body defense mechanism of cattle in which immunity does not act primary by absolute prevention of maturation of challenge infection, but mainly by suppression of worm fecundity. Additionally, once animals are infected with *Giardia*, they become unable to efficiently utilize their feed for maintaining good body condition. Rather, most of the feed content is released in the form of diarrhea as *Giardia* hinders the absorption capacity of intestine. As described by Olson *et al*. [40], *Giardiasis* in domestic ruminants has a negative effect on performance, resulting in decreased rate of weight gain and impaired feed efficiency. As a result, if an animal is infected with *Giardia*, it is most likely true that the animal is unable to maintain its normal body condition.

The result indicated that there was strong statistically significant association between the consistency of the fecal sample and *Giardia* infection (p=0.001). Consequently, the prevalence of Giardia infection was almost sevenfold in diarrheic fecal samples compared to non-diarrheic (OR=7.66, CI=2.25-26.11).

CONCLUSION

Giardiasis is a protozoan disease caused by *Giardia* species that can cause gastrointestinal infection in humans and in a wide variety of animals including farm animals, dog, cat and wild mammals worldwide. In this study 6 major factors were included to be studied with the risk of infection with *Giardia*. These include management system of farms, cattle breeds, sex, age category, body condition status and fecal consistency of the animals sampled. The rate of identification of *Giardia* cyst in cattle was statistically significant (P<0.05) between age groups (< 2 months, 2-6 months and above 6-month age), diarrheic and non-diarrheic cattle and among cattle with good, Medium and poor body condition. But, there was no significance difference in the prevalence rate of *Giardiasis* between the management system of farms (intensive and semi

intensive), cattle breeds (crossbred and local breed) and males and females. Based on the above findings, the following recommendations were forwarded:

- Further studies on *Giardia* using a larger sample size of calves need to be undertaken
- Study involving molecular techniques are required for the identification of the *Giardia* species
- Creation of public awareness on the possible zoonotic existence of *Giardia species* is essential

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