Acta Parasitologica Globalis 13 (3): 73-80, 2022
ISSN 2079-2018

DOI: 10.5829/idosi.apg.2022.73.80

Survey of Intestinal Protozoan Parasites of Dairy Farms in Bishoftu Town, Oromia, Ethiopia

Dinka Ayana, Bethel Befekadu, Bersissa Kumsa and Gebayehu Alkadir

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

Abstract: A cross sectional study was conducted to estimate the prevalence of major intestinal protozoan parasites of dairy cattle in Bishoftu town. A total of 334 fecal samples were collected from private dairy farms, Ethiopia meat and dairy industry development institution (EMDIDI), Ethiopian institute of agricultural research (EIAR) Bishoftu and Addis Ababa University (AAU) business enterprise farm. The samples were processed using fecal flotation, modified Ziehl-Neelsen and formalin ether concentration techniques. From these samples, 218(65.3%) animals were found to be positive to one of the three intestinal protozoan parasites. Three protozoan parasites namely *Eimeria* (53.8%), *Cryptosporidium* (21.9%) and *Giardia* (10.2%) were identified. There was significant difference ($p<0.05$) in the prevalence of *Eimeria* and *Cryptosporidium* parasites between the age group of the study animals. Similarly, there was a significant difference in the prevalence of the parasites between fecal consistency with high prevalence in animal with loose feces. However, there was no significant difference in *Giardia* infections between the age groups, sex and fecal consistency. This study indicates that intestinal protozoan parasites are wide spread in Bishoftu dairy farms so that much attention must be given towards the control and prevention of these parasites.

Key words: Bishoftu · *Cryptosporidium* · Dairy Farms · *Eimeria* · *Giardia* · Prevalence

INTRODUCTION

In Ethiopia livestock production plays an important role to human health and mitigation of poverty. Cattle production has multi-purposed role in this country like for instance milk, meat, fertilizer, fuel, draft power and also as a means of economic uplift from the sale of milk and milk products. This sector contributes 15 to 17% of GDP and 35 to 49% of agricultural GDP and 37 to 87% of the household incomes [1]. But the country's great livestock potential is not properly utilized due to many prevailing socio economic values and customs, limited genetic potential and rampant disease [2].

Gastrointestinal parasites are recognized as by for the most significant part of diseases in livestock sector [3]. Damages inflicted to the health and productivity includes loss in body weight, poor reproductive performance, digestive disturbance, and emaciation for longer period [4]. It has been established that parasitic infestation results in considerable losses in milk production in cattle [5].

Ruminants 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 domesticated cattle, or because of their potential for zoonotic transmission [6].

Eimeria, Cryptosporidium and *Giardia* are genera of protozoon parasites which infect a wide range of vertebrates including domesticated animals and humans [7]. *Cryptosporidium* and *Giardia* have zoonotic importance because they could be transmitted to humans via any mechanism by which material Contaminated with feces containing infectious oocysts can be swallowed by a susceptible host [8].

Coccidiosis is one of the most pathogenic intestinal diseases caused by different species of *Eimeria* belonging to phylum-apicomplexa [9]. The disease is particularly a problem of confined animals that kept under intensive husbandry practices and is more common in housed animals than in those on pastures. In associations with other enteropathogens, coccidia have been indicated as an important cause of diarrhea in calves [4].

Corresponding Author: Dinka Ayana, Department of Pathology and Parasitology, College of Veterinary Medicine and Agriculture, Addis Ababa University, Oromia, Ethiopia.

Many species of *Eimeria* have been described to infect cattle and causes of coccidiosis. *Eimeria bovis* and *Eimeria zuernii* are known to be highly pathogenic eimerian species in cattle worldwide, causing morbidity and even mortality associated with diarrhea, mucus and blood stains [10].

Cryptosporidia are protozoan parasites historically classified as belonging to phylum Apicomplexa, class Coccidea, together with *Eimeria* [11]. Cryptosporidiosis is an emerging protozoan disease, caused by *Cryptosporidium* species, that can cause gastrointestinal infection in a wide variety of mammals including human, cattle, sheep, goat, pig and horses worldwide [12, 13]. *Cryptosporidium parvum* in the intestine and *C. andersoni* in the abomasum cause cattle cryptosporidiosis [7]. Cryptosporidiosis can be asymptomatic or cause pasty to watery and profuse diarrhoea, dehydration, inappetence and even mortality [14].

Giardia is a ubiquitous intestinal flagellated protozoan parasite of mammals. The species *G. duodenalis* is found in humans and various mammalian species [15]. *Giardia* has emerged as an important parasite of dairy cattle [16]. Furthermore, it has been revealed that *Giardia* infections are very common in calves [17].

Very few reports are available on the occurrence of intestinal protozoan infection in cattle in Ethiopia. Therefore, this study was initiated with the objectives to estimate the prevalence of major intestinal protozoan parasites in Bishoftu dairy farms and to identify the associated risk factors with the occurrence of the parasites.

MATERIALS AND METHODS

Study Area: This study was conducted in selected dairy farms of Bishoftu town which is located in the East Shewa zone of Oromiya regional state at 47 km South East of Addis Ababa on the main road to Adama. The precise location of Bishoftu is 8°45'N latitude and 38°59'E longitude. Topographically, the town is located in tepid to cool sub-moist mid highland at an altitude of about 1920 meters above sea level with moderate weather condition. The temperature falls within a range of 16°C and 24°C. The town's total human population is 200,114, of whom 47.1% are maleas reported by 2007 population and housing censes. There are 40 small to large scale dairy farms in Bishoftu town. Intensive management system is practiced in the dairy farms [18].

Study Animals: A total of 334 Holstein Frisian and some cross breed cattle which were classified as young (<2 years) and adult (>2 years) were considered as study animals. The consistency of feces was also categorized as normal, loose, and diarrheic by physical observation.

Study Design: A cross sectional study design was used to estimate the prevalence of *Eimeria, Cryptosporidium and Giardia* parasites in selected dairyfarms.

Sampling Methodology

Sampling Strategy: Cluster sampling technique was used to collect fecal samples from different dairy farms of Bishoftu and hence, all animals in the selected dairy farms were sampled for the study.

Sample Size Determination: Sample size was calculated with an expected prevalence of 68.1% from the previous research work on epidemiology of *Eimeria* infection in calves in Addis Ababa and DebreZeit farms [19]. The desired sample size for the study was determined by using the formula described by Thrusfield [20] as follows:

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

n = required sample size

d = desired absolute precision

P exp = expected prevalence 1.96^2 = z- value for 95% confidence interval. Accordingly, 334 animals were sampled for this study.

Sample Collection and Transportation: Fecal samples were directly collected from the study animals for immediate processing as soon as possible. Different materials like ice box and rectal gloves were used to collect and transport the samples. At the time of collection, labeling was done for individual animal. Then the samples were transported to CVM parasitology laboratory for further processing in the same day of the collection. The sample left unprocessed in the first day was stored in refrigerator at 4°C to be processed in the following days.At the time of sampling, the name of the farm, date of sampling, age, sex, breed and consistency of the feces (loose or normal) were recorded for each individual animal on a data recording format.

Sample Processing: Three laboratory test processes namely direct fecal flotation, Modified Ziehl-Neelsen Stain and Formalin ether concentration techniques were used to identify the oocyst of Eimeria*, Cryptosporidum* and cysts of *Giardia parasites.*

Fig. 1: Map showing the study area

Fecal flotation technique was done by using Sheather's sugar solution to identify the oocysts of *Eimeria.* The coprological procedure is described as follows: taking three grams of feces from each animal sample and mix with 42 ml of flotation fluid by using pistil & mortem thoroughly then the suspension pour through tea strainer. After strain, the suspension was poured in to test tube and the test tube was placed in a rack and the test tube with the suspension was left a convex meniscus at the top of the test tube and coverslip was carefully placed at the top of the tube and let it to stand for 20 minutes. Finally, the coverslip was removed with the drop of fluid adhere to it and immediately the coverslip was put on microscopic slide and examined in 40x magnification to identify *Eimeria* oocysts [19].

Modified Ziehl-Neelsen technique was employed to detect the oocysts of *Cryptosporidium*. The technique is briefly presented as follows: Fecal smears were made directly from the stool samples and the smears were allowed to air dry; the smears were fixed in methanol for 3 minutes. Then the primary stains carbolfuchsin which has red color was added to the smears to stain the smear for 15 minutes. After rinsing thoroughly with tap water, decolorization was done by sulfuric acid for 20 seconds then Counter stain with methylene blue for 1 minute and was rinsed in tap water. Finally, the smears were rinsed thoroughly with water, air dried, and examined using x40 objective [19].

Formalin ether concentration is a technique used to identify *Giardia* cyst.One gram of feces was measured and about 7ml of formalin was added and mixed together and then sieved. The mixture was then transferred to centrifuge tube and on the top 3ml of diethyl ether was added and hand shaken. The content was centrifuged at 2000 rpm for 3minuts and the supernatant was poured away and a drop of sediment was transferred to microscopic slide. One drop of Lugol's iodine solution was added to the sediment on the slide and covered with coverslip. Finally, the entire area under the coverslip was systematically examined using 40x objective lense.

Data Analysis: The entire collected raw data were entered into Microsoft Excel spread sheet and coded. Statistical analyses were performed using STATA version 11 software. Percentage was used to calculate the prevalence. Additionally,Chi square test was used to measure the association and degree of association between risk factors and prevalence of protozoan parasites. In the analysis, a difference was taken as statistically significant at a p-value less than 0.05 and finally results were displayed in the form of tables and graph.

RESULTS

Overall Prevalence of Major Protozoan Parasites: Out of 334 faecal samples examined, 218(65.3%) dairy cattle were found to be positive for at least one of thethree protozoan parasites.

Prevalence of Major Intestinal Protozoan Parasites by the Risk Factors: Statistically, significant difference (p = 0.026) was observed in the parasite infections between the age groups with higher prevalence in the young age category (75.3%) than the adult ones (61.8%) (Fig. 2).

There was also statistically significant difference (p=0.047) in the prevalence of *Cryptosporidium*with regard to fecal consistency. Higher prevalence was recorded in animals with loose feces (70.5%) than normal feces (60.1%) (Fig. 2).

Prevalence of *Eimeria* Parasite by Risk Factors: Out of 334 faecal samples examined 178 dairy cattle (53.3%) were found to be positive for *Eimeria*infection (Table 1).

Prevalence of Cryptosporidium by Risk Factors: Out of 334 faecal samples examined, 73 dairy cattle (21.9%) were positive for *cryptosporidium* parasites (Table 2).

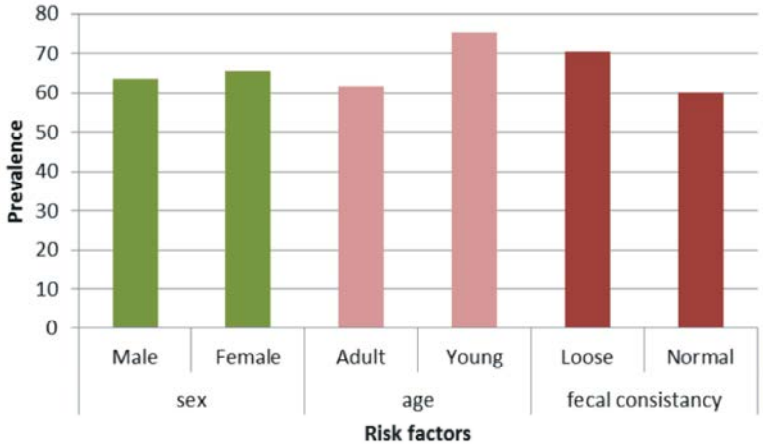

Fig. 2: The overall prevalence of intestinal protozoan parasites by risk factors

Table 1: Prevalence of *Eimeria* parasite by risk factors

Risk factors		No examined	No Positive	Prevalence (%)	χ^2	p-value
Sex	Male	44	22	50	0.22	0.638
	Female	290	156	53.8		
	Total	334	178	53.3		
Age	Adult	249	124	49.8	4.86	0.029
	Young	85	54	63.5		
	Total	334	178	53.3		
Fecal consistency	Loose	166	89	53.6	0.01	0.907
	Normal	168	89	53		
	Total	334	178	53.3		

Table 2: Prevalence of *Cryptosporidium* by the risk factors

Risk factors		No examined	No Positives	Prevalence (%)	χ^2	p-value
Sex	Male	44	14	31.8	2.73	0.090
	Female	290	59	20.3		
	Total	334	73	21.9		
Age	Adult	249	47	18.9	4.84	0.025
	Young	85	26	30.6		
	Total	334	73	21.9		
Fecal consistency	Loose	166	51	30.7	15.52	<0.001
	Normal	168	22	13.1		
	Total	334	73	21.9		

Table 4: Prevalence of *Giardia* infection by risk factors

Risk factors		No examined	No Positives	Prevalence (%)	χ^2	p-value
Sex	Male	44	6	13.6	0.61	0.418
	Female	290	28	9.7		
	Total	334	34	10.2		
Age	Adult	249	27	10.8	0.49	0.494
	Young	85	7	8.2		
	Total	334	34	10.2		
Fecal consistency	Loose	166	18	10.8	0.16	0.690
	Normal	168	16	9.5		
	Total	334	34	10.2		

Prevalence of *Giardia* by Risk Factors: Out of 334 faecal samples examined, 34 dairy cattle (10.2%) were positive for Giardia parasite (Table 4).

DISCUSSION

Among the intestinal protozoan parasites; *Eimeria*, *Cryptosporidium* and *Giardia* have a massive effect on the health and productivity of dairy cattle. These parasites cause diarrhoea and loss of body weight and two of them; *Cryptosporidium* and *Giardia* have zoonotic importance. There was statistically significant difference (P?0.05) in the infections of *Eimeria* and *Cryptosporidium* between the age groups of the study animals. This is in line with the work of Abebe *et al.* [19], Dong *et al.* [21] and Heidari *et al.* [22]. The reason behind might be acquired immunity with host age has shown to cause a difference in an infection [23] and young cattle may be attributed to lower resistance to *Eimeria* species. Previous exposure may also have contribution to the development of a certain level of immunity in older animals as compared to the younger ones [24].

There was a significant difference in the prevalence of *Cryptosporidium* infection between the fecal consistency. However, there was no significant difference in the infection of *Giardia* across the assumed risk factors.

The overall prevalence of *Eimeria* in this study was 53.3% and it was found to be lower than 68.1% as reported by Abebe *et al.* [19] and 87.8% by Rodriguez-Vivas [25]. However, the present finding is higher than 22.7% which was reported by Dawid *et al.* [24] and 24.9% by Kassa *et al.* [26]. The differences in prevalence could be attributed to many factors such as the number of ingested oocysts, the presence of a concurrent microbial infection, weather conditions and seasons, management, the level of immunity and age of the study animals, methods of diagnosis and agro-ecology [27, 28].

There was no statistically significant difference in *Eimeria*, *Cryptospoidium* and *Giardia* infection between male and female animals. This result agrees with the work of Dawid *et al.* [24] indicating that both sexes have an equal chance of being infected with the oocyst and cysts of the parasites.

There was no statistically significant difference in the prevalence of *Eimeria* between fecal consistency ($p > 0.05$). This result is in agreement with the report by Ibrahim *et al.* [29] but it disagrees with the report by Bangoura *et al.* [10]. The difference could be explained that *Eimeria* infection could occur mostly in a subclinical form and the consistency of the feces may be related to diet or other infections.

The overall prevalence of *Cryptosporidium* in this study was 21.9%. This prevalence was relatively similar with that of Ayinmode and Fagbemi [30] (23.4%) in Nigeria and 19.7% in Tanzania by Swai *et al.* [45]. But it was slightly higher than the reports of Abebe *et al.* [19] (17.6%) and by Dinka, Ayana and Berhanu, Alemu [46] (14%) and low relatively compared to the one reported from Nigeria 37.5% by Akinkuotu *et al.* [32] and 32.3% which was reported by Ayinmode and Fagbemi [30]. The variation in prevalence might be due to management system, housing, agro ecological and climatic conditions.

There was no significant difference ($P > 0.05$) in the prevalence of *Cryptosporidium* between the sex of the animals. This is in line with the report of Ayinmode and Fagbemi [30] but our finding contradicts with the report of [31].

There was significant difference ($p<0.05$) in the prevalence *Cryptosporidium* between the age categories of the study animals in which the higher prevalence was recorded in young animals than the adult ones. This is in agreement with the report by Akinkuotu *et al.* [32] and Current and Garcia [33]. Thismight be due to lower immunity by young animals as a result of lack of previous exposure to the parasite. But this result is not in line with the report of Sylvia *et al.* [34].

There is a significant difference ($P = 0.000$) in *Cryptosporidium* infection between fecal consistency with higher prevalence in loose feces. This result is in line

with the report of Sevinc *et al.* [35] and Ouchene *et al.* [36]. This could be justified with the fact that *cryptosporidium* infection is mostly known for causing diarrhea and management problem in the dairy farms could also be the reason. However, this finding is not in agreement with the reports by Ayinmode and Fagbemi [30] and Akinkuotu *et al.* [32].

The prevalence of *Giardia* parasite in cattle ranges from 9 to 73% as reported by Olson *et al.* [37] and Mcallister *et al.* [38]. In this study, the overall prevalence of *Giardia* parasite was 10.2%. This was slightly similar with the reports of 12.2% by Khan *et al.* [39] and 12.25% by Tim *et al.* [40]. The current result was higher than 4.1% which was reported by Degerli *et al.* [13].

In this study, no significant difference was observed between sexes in the prevalence of *Giardia*. But higher prevalence was observed in male than female. The study in Bangladesh by Suman *et al.* [41] also supports this study. This could be due to less care to male animals than that of female animals. However, other studies in dairy cattle revealed more females were infected with *Giardia* than males [42].

There was no significant difference in the prevalence of the *Giardia* between age groups of the study animals. This finding agrees with the study by Olson *et al.* [37]. But the result disagrees with the findings by Khan *et al.* [39] and Huetink *et al.* [43].

There was no significant difference in the prevalence of *Giardia* between fecal consistencies.This could be due to the chronic intermittent diarrhoea episodes that are associated with *Giardia* infections so much that one-time sampling may be unable to detect an association [44]. This study agrees with the report of Huetink *et al.* [43] who did not find an association between presence of *Giardia* cysts in faecal specimens and change of feces in individual hutch calves or adult cattle. But some studies like that of Mcallister *et al.* [38] indicated that*Giardia* infection and consistency of the feces are significantly associated.

CONCLUSION AND RECOMMENDATION

During this study, high prevalence of intestinal protozoan parasites was registered in Bishoftu dairy farms. Significant difference in Eimeria and *Cryptosporidium* infections was observed between the age groups of the study animals with higher prevalence in the young age group. There was also a significant difference in the infection between the feces type with higher prevalence in loose feces. The majority of positive cases in this study were clinically asymptomatic which could result in a serious economic loss to the dairy industries. Further studies based on advanced laboratory techniques like molecular analysis should be conducted to identify the species /genotypes of the parasites circulating in the dairy farms. The burden of the infections by *Eimeria, Cryptosporidium* and *Giardia* parasites should also be conducted and the economic impacts due to sub clinical infections by these parasites need to be studied.

REFERENCES

1. Behnke, R. and F. Metaferia, 2011. The Contribution of Livestock to the Ethiopian Economy-Part II, IGAD Livestock Policy Initiative (LPI) Working Paper No. 02-11, Retrieved on September 20, 2013.
2. Debela, E., 2002. Epidemiology of gastro-intestinal helminthiasis of Rift Valley goats under traditional husbandry system in Adami Tulu district, Ethiopia. Eth. J. Sci., 25: 35-44.
3. Waller, P.J., 1999. International approaches to the concept of integrated control of nematodes parasites of livestock, Int. J. Parasitol., 29: 155-164.
4. Radostits, O.M., D.C. Blood and C.C. Gay, 1994. Diseases caused by helminth parasites. In: Veterinary Medicine: a textbook of diseases of cattle, sheep, pigs, goats and horses, 8th Edition. London, Balliere Tindall, pp: 1223-1230.
5. Hayat, C., B. Hayat, M. Ashfaque and K. Muhammad, 1984. Bottle jaw in Berberi (Teddy) goat. Pak. Vet. J., 4: 183.
6. Mike, A. and Taylor, 2000. Protozoal disease in cattle and sheep. In practice, 22(10).
7. Caccio, S., T. Andrew, J. Mclauchlin and H. Smith, 2006. Unravelling Cryptosporidium and Giardia epidemiology. Trends Parasitol., 21: 43-437.
8. Smith, H.V., 2004. Cryptosporidiosis, Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Organisation Internationale des Epizootes–OIE, Paris, France, pp: 1082-1100.
9. Almeida, V.D., V.C. Magalhaes, E.S. Muniz-Neta and A.D. Munhoz, 2011. Frequency of species of the genus Eimeria in naturally infected cattle in Southern Bahia, Northeast Brazil. Braz. J. Vet. Parasitol., 20: 78-81.

10. Bangoura, B., H. Mundt, R. Schmäschke, B. Westphal and A. Daugschies, 2012. Prevalence of Eimeriabovis and Eimeriazuernii in German cattle herds and factors influencing oocyst excretion. Parasitological Research, 110: 875-881.
11. Fayer, R., 2008. General Biology. In: Fayer, R. & Xiao, L. (Eds.) Cryptosporidium and cryptosporidiosis, 2nd ed. Boca Raton: CRC Press, pp: 1-42.
12. Nasir, A., M. Avais, M. Khan and N. Ahmad, 2009. Prevalence of Cryptosporidium parvum infection in Lahore (Pakistan) and its association with diarrhea in dairy calves. Int. J. Agric. Biol., 11: 221-224.
13. Degerli, S., A. Celiksoz, K. Kalkan and S. Ozcelik, 2005. Prevalence of *Cryptosporidium* Spp. and *Giardia* Spp. in cows and calves in Sivas. Turk J. Vet. Sci., 29: 995-999.
14. Charlotte, Silverlås, 2010. Cryptosporidium Infection in Dairy Cattle; Doctoral Thesis, Swedish university of agricultural sciences, Faculty of Veterinary Medicine –Sweden.
15. Thompson, R.C., R.M. Hopkins and W.L. Homan, 2000. ‘‘Nomenclature and genetic groupings of Giardia infecting mammals.” Parasitology Today, 16: 210-213.
16. Olson, M.E., R.M. O’handley, B.J. Ralston, A.T. Mcallister and R.C. Thompson, 2004. Update on Cryptosporidium and Giardia infections in cattle. Trends in Parasitology, 20(4): 185-191.
17. O’Handley, R., M. Olson, D. Fraser, P. Adams and R. Thompson, 2000a. ‘‘Prevalence and genotypic characterization of Giardia in dairy calves from Western Australia and Western Canada.” Veterinary Parasitology, 90: 193-200.
18. Birhanu, M., L. Seta, G. Mamo and S. Tesfaye, 2017. Prevalence of bovine subclinical mastitis and isolation of its major causes in Bishoftu Town, Ethiopia. BMC Res Notes. 10: 767.
19. Abebe, R., A. Wossene and B. Kumsa, 2008. Epidemiology of Eimeria Infections in Calves in Addis Ababa and DebreZeit Dairy farms, Ethiopia. Intern. J. Appl. Res. Vet. Med., 6: 24-30.
20. Thrusfield, M., 2007. Veterinary Epidemiology. rd Ed. Blackwell Science Ltd., Oxford, UK, pp: 233-261.
21. Dong, H., Q. Zhao, H. Han, L. Jiang, S. Zhu, 2012. Prevalence of coccidial infection in dairy cattle in Shanghai, China. Journal of Parasitology, 98: 963-966.
22. Heidari, H., Z. Sadeghi-Dehkordi, R. Moayedi and J. Gharekhani, 2014. Occurrence and diversity of Eimeria species in cattle in Hamedan province, Iran. Veterinarni Medicina, 59: 271-275.
23. Yu, S., M. Gao, N. Huang, Y. Jia and Q. Lin, 2011. Prevalence of coccidial infection in cattle in Shaanxi province, Northwestern China. Journal of Animal and Veterinary Advances, 10: 2716-2719.
24. Dawid, F., Y. Amede and M. Bekele, 2012. Calf Coccidiosis in selected dairy farms of Dire Dawa, Eastern Ethiopia. Glo. Vet., 9: 460-464.
25. Rodriguez-Vivas, R.I., J.L. Dominguez-Alpizar and J.F. Torres-Acosta, 1996. Epidemiological factors associated to bovine coccidiosis in calves (Bosindicus) in a sub humid tropical climate. Revista Biomédica, 7: 211-218.
26. Kassa, B., A. Delgad and T. Asegedech, 1987. An outbreak of coccidiosis in cattle. Ethiop. Vet. Bull., 3: 20-27.
27. Warui, R., N. Kyvsgard, S. Thamsb-Borg, P. Nansen, H. Bogh, W. Mundau and J. Gathuma, 2000. The prevalence and intensity of helminth and coccidial infections in dairy cattle in central Kenya. Vet. Res. Comu., 24: 39-53.
28. Radostits, O.M., C.C. Gay, K.W. Hinchcliff and P.D. Constable, 2007.Veterinary Medicine, A text book on disease of cattle, sheep, pigs and horse, 10th ed. Edinburgh: Saunders.
29. Ibrahim, M.M., M. Soliman and A.O. Alghamdi, 2015. Subclinical Bovine Coccidiosis in Al –Baha Area, Saudi Arabia. Int. J. Vet. Sci. Res., 1(1): 023-028.
30. Ayinmode, A.B. and B.O. Fagbemi, 2010. Prevalence of Cryptosporidium infection in cattle from South Western Nigeria. Vet. Arhiv., 80: 723-731.
31. Maikai, B., J. Umoh, J. Kwaga, I. Lawal, V. Maikai, V. Camae and L. Xiao, 2011. Molecular characterization of Cryptosporidium spp. in native breeds of cattle in Kaduna State, Nigeria. Veterinary Parasitology, 178(3-4): 241-245.
32. Akinkuotu, O.A., B.O. Fagbemi, E.B. Otesile, M.A. Dipeolu and A.B. Ayinmode, 2014. Cryptosporidium infection in cattle in Ogun state, Nigeria Sokoto Journal of Veterinary Sciences, 12 (Number 2).
33. Current, W.L. and L.S. Garcia, 1991. Cryptosporidiosis. Clin. Microbiol. Rev., 4: 325-258.
34. Sylvia, A., B. Julius and A. Hamphery, 2013. Prevalence of Cryptosporidium oocysts in cattle from Southern Ghana VETERINARSKI ARHIV, 83(5): 497-507.
35. Sevinc, F., K. Irmak and M. Sevinc, 2003. The prevalence of Cryptosporidium parvuminfection in the diarrhoiec and non- diarrhoeic calves. Rev. Méd. Vét., 154(5): 357-361.

36. Ouchene, N., N. Ouchene-Khelifi, F. Zeroual, A. Benakhla and K. Adjou 2014. Study of Giardia spp., *Cryptosporidium* spp. and *Eimeria* spp. infections in dairy cattle in Algeria. Journal of Parasitology and Vector Biology, 6(4): 61-65.
37. Olson, M.E., N.J. Guselle, R.M. O'Handley, M.L. Swift, T.A. Mcallister, M.D. Jelinski and D.W. Morck, 1997. Giardia and Cryptosporidium in dairy calves in British Columbia. Can. Vet. J., 38: 703-706.
38. Mcallister, T., M. Olson, A. Fletch, M. Wetzstein and T. Entz, 2005. Prevalence of Giardia and Cryptosporidium in beef cows in southern Ontario and in beef calves in southern British Columbia. Can. Vet. J., 46: 47-55.
39. Khan, S., C. Debnath, A. Pramanik, L. Xiao, T. Nozaki and S. Ganguly, 2011. ''Molecular evidence for zoonotic transmission of Giardia duodenalisamong dairy farm workers in West Bengal, India." Veterinary Parasitology, 178: 342-345.
40. Tim, A., M. McAllister, A. Olson, W. Mer and E. Toby, 2005. Prevalence of Giardia and Cryptosporidium in beef cows in southern Ontario and in beef calves in southern British Columbia Can Vet. J., 46(1): 47-55.
41. Suman, M., M. Alam, S. Pun, A. Khair, S. Ahmed and R. Uchida, 2011. ''Prevalence of Giardia lambliainfection in children and calves in Bangladesh." Bangladesh Journal of Veterinary Medicine, 9: 177-182.
42. Ayaz, S., A. Maqbool, S. Khan, S. Khan, M. Hussian, N. Akbar, R. Khan and J. Khan, 2012. ''Prevalence of giardiasis in selected dairy cattle farms in Lahore, Pakistan, African Journal of Microbiology Research, 6: 5383-5386.
43. Huetink, R., J. Van Der Giessen, J. Noordhuizen and H. Ploeger, 2001. Epidemiology of *Cryptosporidium* spp. and Giardia duodenalis on a dairy farm" Veterinary Parasitology, 102: 53-67.
44. O'Handley, R., H. Ceri, C. Anette and M. Olson, 2003. ''Passive immunity and serological immune response in dairy calves associated with natural Giardia duodenalis infections, Veterinary Parasitology, 113: 89-98.
45. Swai, E., N. French, E. Karimuribo, F. Jitzpatrick, M. Bryant, D. Kambarage and N. Ogden, 2007. Prevalence and determinants of Cryptosporidium spp.infection in smallholder dairy cattle in Iringa and Tanga Regions of Tanzania. Onderstepoort J. Vet. Res., 74: 23-29.
46. Dinka, Ayana and Berhanu, Alemu, 2015. Cryptosporidiosis in Calves, Lambs and Goat Kids in Bishoftu, Oromia Regional State, Ethiopia African Journal of Basic & Applied Sciences, 7(5): 233-239.