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Study on Eimeria and Cryptosporidium Infection in Dairy Cattle Farms of Holeta, West Shoa Zone, Oromia, Ethiopia

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Abstract: A cross-sectional study was conducted from November, 2017 up to April, 2018 in and around Holeta dairy farms, West shoa zone, Wolmera district, Oromia, Ethiopia to determine the prevalence of eimeriosis and cryptospordiosis in dairy cattle and to assess the associated risk factors. Three hundred seventy eight (378) fecal samples were collected from dairy cattle and examined for the presence of the oocysts of Eimeria by floatation technique using concentrated sucrose solution and for that of oocysts of Cryptosporidium employing Modified Ziehl Nelseen Acid fast staining technique. The study revealed that the overall prevalence of eimeriosis and cryptospordiosis was 51.9%. However, the prevalence of Eimeria and Cryptosporidium was found to be 47.1% and 10.8%, respectively. In this study age, sex, production system, body condition and fecal consistency were considered as risk factors. There was a statically significant difference ($P<0.05$) in the overall prevalence of Eimeria and Cryptosporidium oocysts among animals with different age and fecal consistency. In the current study, there was statistically significant difference ($P<0.05$) in the prevalence of eimeriosis between the different age groups with the highest prevalence being recorded in age category less than 6 months (58.3%). However, the overall prevalence of Cryptosporidium positive dairy cattle was 10.8%. Prevalence of cryptosporidium infection in calves (less than 6 month), young (7-24 month) and adult (>24 month) cattle were 25%, 12.8% and 4.2%, respectively. The age specific prevalence was found to be statistically significant ($P<0.05$) with Cryptosporidium infection. The highest prevalence of the Cryptosporidium infection was recorded in animals with diarrheic feces (25.6%) than animals with non- diarrheic fecal consistency ($P<0.05$). The present study indicated that both Eimeria and Cryptosporidium infection were prevalent in Holeta, especially in calves less than 6 month age, poor body condition, diarrheic, male and those dairy cattle managed intensively. In general, the results demonstrated the presence of these parasites and underlined the significance of sub clinical coccidiosis in adult animals in the study area which urgently addressed to counteract the negative impact on productivity and health of animal. Moreover, the results of the current study revealed the importance of the two protozoal parasites (Eimeria and Cryptosporidium) among animals in dairy farms requiring a serious attention towards control and prevention.

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1. Introduction

Ethiopia is endowed with abundant livestock resources of varied and diversified genetic roles with specific adaption to its wide range of agroecologies (CSA, 2015). The economy is predominantly based on agriculture which is considered as a primary factor in securing food self-sufficiency, generating employment and income for the poor. Livestock sub sector plays a vital role which contributes 33% of agricultural gross domestic product (GDP) and 19% to the export earnings. In addition, crop production is almost exclusively dependent on livestock especially draft power of cattle. Cattle production, among the sector of livestock production systems, is a critical issue in Ethiopia. In spite of all this, full exploitation of cattle potential is mainly constrained and impeded at a great extent by parasitic diseases (Zegeye, 2003).

The infection with various types of gastrointestinal parasites in cattle is a worldwide problem (Dorny *et al*., 2011). Gastrointestinal (GI) parasitic infections may be considered as one of the major constraints in cattle production. The infection causes productivity losses through reduced feed intake and decreased efficiency in feed utilization due to subclinical or chronic infections that are responsible for economic losses. GI parasitic infections in cattle in general cause economic losses to the livestock owner

due to decreased milk production (Bandyopadhyay *et al.*, 2010).

Coccidiosis is a parasitic disease of the intestinal tract caused by Eimeria and is one of the most common and important disease of cattle worldwide. Bovine coccidiosis is considered to be of considerable importance for the productivity and health of cattle. All age groups of cattle are susceptible to infection, but clinical Eimeriosis is most common in young animals. Coccidiosis in cattle commonly occurs as subclinical disease without signs of the disease and involving great economical losses due to reduced appetite, reduced body weight, impaired feed conversion, unthrift, diarrhea, dysentery, anemia, and increased susceptibility to other diseases (Bahrami and Alborzi, 2013). Infected calves are also more susceptible to secondary diseases, such as pneumonia, bacterial enteritis and viral infections (Daugschies and Najdrowski, 2005).

Eimeria species have been identified to cause disease in a range of animals (Pigs, poultry and lambs), however they are host specific (i.e., Cattle Eimeria spp. cannot infect sheep) and 13 species have been isolated from cattle (von Samson-Himmelstjerna *et al*., 2006). 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 (Bangoura *et al*., 2012).

Infections can occur both indoors, on damp and faeces contaminated bedding, or outdoors, around drinking and feeding troughs. Low infections levels will result in the induction of protective immunity. Light infections are self-limiting, but severe infections can be fatal if untreated. Oocysts can be seen in faecal samples using light microscopy. However, oocysts counts can sometimes be unreliable as healthy animals can pass more than 10 million oocysts per gram of faeces, animals can die of coccidosis before any oocysts are shed and oocyst output may be transient so an animal that is dying of coccidiosis may show very few oocysts (Taylor, 2000).

As infections are initiated by ingestion of oocysts, control strategies must be aimed at reducing the number of oocysts in the environment. Measures to reduce the risk of infection include the removal of food contaminated with faeces and better placement of feeding and water troughs. If possible, creep feeders should be moved at regular intervals to prevent the buildup of infection around them. Also, if possible, calves should be turned out in the spring to pasture that has not been grazed by calves in the previous year (Svensson, 1997; Svensson, 2000).

Cryptosporidium spp. is also another protozoan parasite that can cause gastrointestinal infection known as cryptosporidiosis. This parasite is responsible for veterinary problems particularly enteric illness outbreaks in livestock animals (Gormley *et al*., 2011). Cryptosporidium infection causes important economic impact to farmers due to its high morbidity and sometimes, high mortality rates in farm animals and sheep and goats (Kaupke *et al*., 2017). In farm animals, cattle are recognized as the most common mammalian species that can be infected by Cryptosporidium (Huang *et al*., 2014). It is known since 1980s that calves and cattle have been identified as an important reservoir for zoonotic Cryptosporidium spp. (Izadi *et al*., 2014). Four species commonly affect cattle; *Cryptosporidium parvum, Cryptosporidium bovis, Cryptosporidium andersoni*, and *Cryptosporidium ryanae* (Brook *et al*., 2008). The major Cryptosporidium spp. that infects calves is *Cryptosporidium parvum* which can cause severe watery, yellowish, and foul smelling acute diarrhea that may lead to morbidity and mortality (Lendner and Daugschies, 2014).

A variety of methods is available for detection of Cryptosporidium species including microscopic, immunological and molecular techniques. Microscopic detection is based on finding the environmental and chemical resistant oocysts in fecal samples. Oocysts may be demonstrated using Ziehl Nielsen stained fecal smears in which the sporozoites appear as bright red granules (Taylor *et al*., 2007). Infected farm animals, particularly cattle, are considered sources of human infection; this concern has put pressure on researchers and farmers to identify and manage the risks associated with the spread of the zoonotic infection (Taylor *et al*., 2007).

Although, eimeriosis and cryptosporidiosis are an important cause of calf morbidity and mortality in Ethiopia in general, and in the study area in particular, there is no previous detail information on prevalence of eimeriosis and cryptospordiosis of dairy cattle in the study area. However, some works have been conducted to determine the prevalence and significance of calf coccidiosis in few areas of the country which include (Bekele *et al*., 2012) 22.7% in Dire dawa, (Abebe *et al.,* 2008) in Debrezeit and Addis Ababa and (Yadessa *et al.,* 2014) 51.7% in Jimma. As a result, there is a scarcity of information on the occurrence and losses associated with bovine eimeriosis and *c*ryptospordiosis and very little attention has been given to the role of coccidiosis as the cause of disease and production losses in cattle in Ethiopia, like in Holeta.

In light with the above, the objectives of this study were to investigate the prevalence of Eimeria and Cryptosporidium parasites in dairy cattle in the

study area and to assess the possible risk factors associated with the disease occurrence in the study area.

2. Materials and Methods

2.1. Study Area

Study was conducted in dairy cattle farms of Holeta selected in random manner. 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^{o}C with a 9°15 N and longitude of 38°25'- 38°45' E. Population in 2015 was 57,828 with an average of 6.7 members per household. The area got annual rain fall in between 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%. 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 (Veses *et al*., 2016). 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 (WoWAHA, 2015).

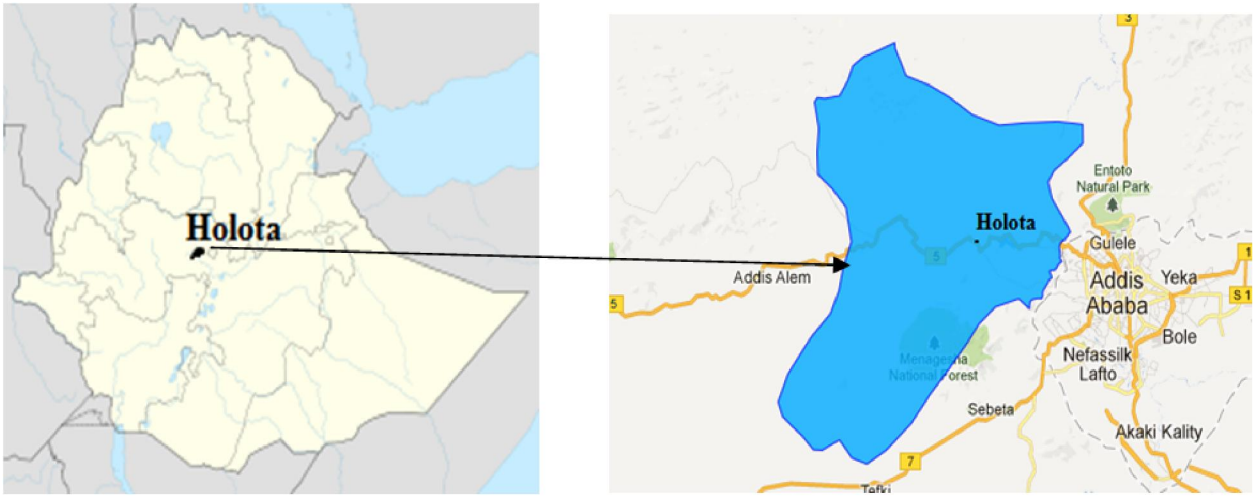


Figure 1. Map of the Study Area (Wolmera District, Holeta) (Ikimap, 2018)

2.2. Selection of Study Animals

The study animals were dairy cattle representing both males and females belonging to the cross breed of Holstein Friesian maintained under traditional small holder and large scale intensive and semi-intensive production system. The study dairy farms were selected purposively based on their potential of animal's population and accessibility. Once dairy farms are identified and sampling frame is established all animals were sampled randomly. Study animals were considered irrespective of age, sex, body condition score and color. The categorization of the study animals into different age groups was based on conventional approach. Accordingly, animals up to 6 months of age were considered as calves and those animals between 6 and 24 months of age were grouped as young animals. Animals above 24 months of age were identified as adults. The age of animals was determined by asking the owner of the animal, looking their ear tag number and dentition criteria (Annex 4). Animals were also examined clinically for evidence of diarrhea. Animals with fluid faeces and/or soiled hind quarters were categorized as diarrheic, while those not manifesting these features were considered to be non-diarrheic.

2.3. Study Design

A cross sectional study was conducted from November 2017 to April 2018 with an involvement of systematic random sampling method from the farms of the study area to determine the prevalence of Eimeria and Cryptosporidium infection and to assess the possible risk factors associated to the disease.

2.4. Sample Size Determination

Sample size was calculated with an expected prevalence of 56.3% from the previous research work on prevalence of coccidiosis on dairy cattle at Holeta Agricultural Research Center Dairy Farm, Ethiopia according to (Getahun *et al*., 2017). The Sample size required for the study was determined using the formula given by Thrustfield (2007) as follows:

$$n=\frac{(1.96)^2 p_{exp}(1-p_{exp})}{d^2}=\frac{(1.96)^2 0.563(1-0.563)}{0.05^2}=378$$

Where; n is the sample size, Z (1.96) is the statistic corresponding to level of confidence 95%, *P* is the expected prevalence and d is precision which was taken as 5%. Therefore, a total of 378 samples were needed.

2.5. Sample Collection and Examination

A fresh fecal sample of about 20gm was collected from the rectum of each animal using sterile disposable plastic gloves and was labeled and kept in icebox and transported to Parasitology laboratory of Addis Ababa university College of Veterinary Medicine and Agriculture. Sample not subjected to examination were kept at 4°C in a refrigerator until processing within 48 hours of arrival. At the time of sampling, the name of the farm (Owner), date of sampling, consistency of the feces (Diarrheic, soft or normal) and the age, sex, address, management system (semi-intensive and intensive) and, body condition (poor, medium, good) of smallholder and large scale dairy farm were recorded for each dairy cattle on a data recording format.

In the laboratory, Qualitative fecal examination was conducted using simple flotation technique for the detection of the oocysts of Eimeria using concentrated sucrose solution (Sheather's sucrose solution) with specific gravity of 1.27 and for that of the oocysts of Cryptosporidium Modified Ziehl Neelsen acid fast staining technique was also performed. Details of the preparation, procedure and protocol of the technique are depicted in (Annex1-3). The Morphological characterization of oocysts of Eimeria and Cryptospordium was done as per the guidelines of (Erastus *et al*., 2012).

2.6. Data Analysis

The entire collected raw data were entered into Microsoft Excel spread sheet and coded. Statistical analyses were performed using SPSS, version 20 software packages. Percentage was used to calculate prevalence. Additionally, chi-square was used to measure degree of association between risk factors and prevalence of eimeriosis and cryptosporidiosis. In the analysis, a difference was taken as statistically significant at a p-value less than 0.05 and the results were displayed in the form of tables.

3. Results

The overall prevalence of eimeriosis and cryptosporidiosis in the study area were found to be 47.1% (178/378) and 10.8% (41/378), respectively. However, the prevalence of mixed infection was about 5.8% (22/378) or 11.2% out of 196 positive samples with the overall prevalence of 51.9% (196/378) (Table 1) in which mixed infections occurred with both Eimeria and cryptosporidium. From examined 72 calves with the age category of less than 6 months, 50 (69.4%) were found positive with either Eimeria or Cryptosporidium. The prevalence of infection with one of these parasites in young animals (117) with the age category between 6 month up to 24 month was found to be 64(54.7%) while the prevalence of infection in adult animals (189) with the age category above 24 months was about 82 (43.4%). The result showed there was also a statically significant difference ($P<0.05$) in the overall prevalence of Eimeria and Cryptosporidium oocysts among animals with different age and fecal consistency (Table 2). Accordingly, the highest prevalence was recorded in those calves up to 6 months old age followed by those calves at 6-24 and above 24 months old age ($P<0.05$) and in diarrheic animals.

Table 1. Overall Prevalence of Eimeria and Cryptospordium

Type of parasite oocyst found	Number examined	Number of positive animals	Prevalence (%)
Eimeria	378	178	47.1%
Cryptosporidium	378	41	10.8%
Mixed (Eimeria +Cryptospordium)	378	22	5.8%
Overall prevalence		**196**	**51.9%**

Table 2. Overall Prevalence of Infection against Associated Variables

Risk factors	No. examined	No. of positive animals	Prevalence (%)	X^2 (P-value)
Sex				
Male	52	27	51.9	
Female	326	169	51.8	0.0001 0.991
Age				
<6 month	72	50	69.4	
7-24 month	117	64	54.7	14.7316 0.001
>24 month	189	82	43.4	
Body condition				
Poor	77	41	53.3	
Moderate	156	80	51.3	0.0812 0.960
Good	145	75	51.7	
Fecal consistency				
Diarrheic	78	46	59.1	
Non-diarrheic	300	150	50	3.9701 0.046
Management system				
Semi-intensive	245	123	50.2	
Intensive	133	73	54.9	0.7573 0.384
Overall prevalence	**378**	**196**	**51.9**	

The prevalence of eimeriosis and cryptosporidiosis by sex: from examined 52 male animals, 25 (48.1%) and 7 (13.5%) were found positive for Eimeria and Cryptosporidium oocysts, respectively and from examined 326 female animals, 153 (46.9%) and 34 (10.4%) were found positive for Eimeria and Cryptosporidium, respectively. Accordingly, the prevalence was higher in male animals as compared with female animals (Table 3 and 4).

Prevalence of eimeriosis and cryptosporidiosis by age**:** from examined 72 calves with the age category of less than 6 months, 42 (58.3%) and 18 (25%) were found positive for Eimeria and Cryptosporidium, respectively. However, the prevalence of Eimeriosis and Cryptospordiosis of young animals (117) with the age category between 6 month up to 24 month were 58 (49.6%) and 15 (12.8%) respectively while the prevalence of Eimeriosis and Cryptosporidiosis in adult animals (189) with the age category above 24 month were about 78 (41.3%) and 8 (4.2%) respectively. Accordingly, the highest prevalence was recorded in those calves up to 6 months old age followed by those calves at 6-24 and above 24 months old age ($P<0.05$) (Tables 3 and 4).

Prevalence of eimeriosis and cryptosporidiosis by fecal consistency: among samples collected, 78 were diarrheic of which 38 (48.7%) and 20 (25.6%) harboured Eimeria and Cryptosporidium parasites, respectively. From 300 non-diarrheic animals, 140 (46.6%) and 21 (7%) were positive for Eimeria and Cryptosporidium, respectively. Accordingly, the higher prevalencee was observed in diarrheic animals (Table 3 and 4).

Prevalence of eimeriosis and eryptosporidiosis by body condition**:** out of 77 dairy cattle's, which were categorized under poor body condition, 39 (50.6%) harboured Eimeria and 12 (15.6%) of them harbored Cryptosporidium oocysts. On the other hand, among 156 animals which were in the category of moderate body condition, 73 (46.8%) and 13 (8.3%) were positive for Eimeria and Cryptosporidium infections, respectively while 66 (45.5%) and 16 (11.1%) animals were positive for Eimeria and Cryptosporidium parasites from145 dairy cattle which were categorized as good in body condition. Accordingly, the highest prevalence was recorded in those cattle with poor body condition (Table 3 and 4).

Prevalence of eimeriosis and cryptosporidiosis by management system**:** among 245 dairy animals which were reared under semi-intensive management system, 111 (45.5%) were harboured Eimeria parasite while 24 (9.8%) were positive for cryptosporidium. However, 67 (50.4%) and 17 (12.8%) of dairy animals from 133 dairy cattle reared by intensive production system were positive for Eimeria and Cryptosporidium parasites, respectively. From the result, higher prevalence was recorded from dairy cattle which were reared by intensive management production system as compared with semi-intensive production system (Tables 3 and 4).

Table 3. Prevalence of eimeriosis against the Associated Variables

Risk factor	No. examined	No. positive	Prevalence (%)	X^2	P-value
Sex					
Male	52	25	48.1		
Female	326	153	46.9	0.0236	0.878
Age					
<6 month	72	42	58.3		
7-24 month	117	58	49.6		
>24 month	189	78	41.3	6.5121	0.039
Fecal consistency					
Diarrheic	78	38	48.7		
Non-diarrheic	300	140	46.6	0.1045	0.746
Body condition					
Poor	77	39	50.6		
Moderate	156	73	46.8	0.5409	0.763
Good	145	66	45.5		
Management system					
Semi-intensive	245	111	45.3		
Intensive	133	67	50.4	0.8893	0.346
Total	**378**	**178**	**47.1**		

Table 4. Prevalence of cryptosporidiosis against the Associated Variables

Risk factor	No examined	No positive	Prevalence (%)	X^2	P-value
Sex					
Male	52	7	13.5		
Female	326	34	10.4	0.4264	0.514
Age					
<6 month	72	18	25		
7-24 month	117	15	12.8	23.936	0.001
>24 month	189	8	4.2		
Fecal consistency					
Diarrheic	78	20	25.6		
Non-diarrheic	300	21	7	22.245	0.000
Body condition					
Poor	77	12	15.6		
Moderate	156	13	8.3	2.8117	0.245
Good	145	16	11.1		
Management system					
Semi-intensive	245	24	9.8		
Intensive	133	17	12.8	0.7949	0.373
Total	**378**	**41**	**10.8**		

4. Discussions

The current study showed the presence of Eimeria and Cryptosporidia oocysts, in calves (<6 month), young and adult dairy cattle. The overall prevalence for both parasites was found to be 51.9%. The prevalence was higher in calves in age category of less than 6 months of age as compared to young and adult cattle for both parasites since calves are born immunologically naïve (immature immunity) (Wymann, 2005) and their immunity matures with time, therefore calves above 6 months of age are able to mount a higher primary immune response than calves less than 6 months of age (Tizard, 2009). Therefore, the development of some immunity could have partially led to the slightly lower prevalence in calves above 6 months. There was also statistically significant difference in the prevalence of the parasitic conditions between diarrheic and non-diarrheic fecal consistency ($P<0.05$). From this study adult animals were subclinicaly infected in some extent as compared

to young and calves. Hence, good management practices should be employed for these calves in particular and all dairy cattle in general. Therefore, the sources of infection were likely similar. Mixed infections were also reported, hence reducing the number of overall infections and thus mixed infections as well.

The overall prevalence of Eimeria and Cryptosporidium oocysts were 47.1% and 10.8%, respectively. The prevalence of eimeriosis in the present study roughly agrees with the previous works reported by (Getahun *et al*., 2017) in Hollota (56.3%), (Dong *et al*., 2012) in china (47.1%) in dairy cattle, (Getrude *et al*., 2015) in kenya mukurwi and (Yadessa *et al.*, 2014) (51.7%) in Jimma in dairy calves. In contrary, the result is lower than previous findings reported in Addis Ababa and Debrezeit by (Abebe *et al*., 2008) (68.1%), 62.5% in Asella town by (Ibrahim, 2016), in the coastal plain area of Georgia (USA) (82.28%) and in sub-humid tropical climate by (Rodriguez-Vivas *et al*., 1996) (87.8%). The result is a bit higher than the previous prevalence reported by (Gillhuber *et al*., 2014) (13.3%) in Southern Germany, Hussin (2016) (9.5%) in Iraq, (Heidari *et al.* 2014) (8.25%) in Iran, (Chibunda *et al.*, 1997) (35%) in Tanzania, (Nagwai *et al*., 2011) in south Africa and (Das *et al*., 2015) (11.9%) in India. Such inconsistency in the prevalence rate of eimeriosis may be due to the variation in diagnostic tests, age of the animals, susceptibility of different breeds to the disease, stress level, climatic and other factors of agroecology, number of ingested oocysts, variation in the study season, number and target group of the study animals and husbandry practices in different places (Heidari *et al*., 2014). Although Eimeria parasites were isolated from our study animals, there was no clinical disease observed on the visits. This finding suggests that most Eimeria infections in these animals were mild or sub-clinical and were more likely important in causing negative animal performance than clinical disease (Rehman *et al*., 2011).

Analysis of risk factor with regard to the sex revealed that there was no statistically significant difference in prevalence of Eimeria infection between male and female animals ($\chi 2$ =0.0236, p =0.878), which is in agreement with the reports of (Abebe *et al*., 2008, Heidari and Gharekhani, 2014, Alemayehu *et al*., 2013, Bekele *et al.,* 2012 and Ibrahim, 2016). This might be due to the fact that different sex groups kept in similar husbandry system might have equal chance of being infected with the oocyts. Yet, a higher prevalence in male animals could be due to the less care given to the male calves as compared to the female calves that are deemed to be future cows and may be due to the size of sample. In spite of this, previous studies done on adult cattle showed higher prevalence of Eimeria in female animals than in males (Rehman *et al*., 2011). This could be due to the physiological stress loaded on female animals in relation to pregnancies and lactation as compared to males (Radostits *et al*., 2007).

In the present study, an age-related difference in the prevalence of Eimeria infection was observed. The lowest prevalence (41.3%) was seen in cattle >24 months old. In contrast, cattle <6 month and between 6 and 24 month in age showed relatively higher prevalence's compared to older animals, 58.3% and 49.6%, respectively. This finding agrees with the report of (Bekele *et al.*, 2012, Almeida *et al*., 2011, Yu *et al*., 2011, Matjila and Penzhorn, 2002, Cicek *et al*., 2007, Rehman *et al*., 2011, Nisar-Khan *et al*., 2013 and Heidari and Gharekhani, 2014). But this study was in contrast of (Abebe *et al*., 2008) who reported that risk of infection by Eimeria species appeared to increase with the age of the examined cattle and a survey conducted in Tanzania showed that coccidial infections were more prevalent in weaners (4-18 mo) than in unweaned calves (<4 months) and adults (>18 months) (Chibunda *et al.,* 1997). However, it has been stated that eimeriosis is commonly a disease of young cattle 1-2 months to 1 year (Radostitis *et al.*, 1994). In the current study, young animals were excreting the highest numbers of oocysts. The infection intensity of cattle <6 months old was significantly higher ($P < 0.05$) than in 6-24 months old and >24-months old cattle, which is consistent with previous observations (Matjila and Penzhorn, 2002). Thus, stress factors like weaning, change of diet, sanitation and overcrowding can increase level of infection (Johannes, 1996). Increasing prevalence rate in low age groups may also be due to immature immune system and their high sensitivity to infection (Matjila and Penzhorn, 2002). And also previous exposure might have a contribution to the development of certain level immunity of older calves as compared to younger that did not experienced previous exposure (Faber *et al*., 2002) resulting in more susceptibility to coccidiosis than older calves with immunity from previous exposure. The possibility of adult animals acting as a reservoir for younger ones in stall fed conditions is also an added explanation (Abebe *et al*., 2008).

There was no statistically significant ($P>0.05$) difference in prevalence rate between fecal consistency and Eimeria infection which disagrees with the findings of (Pandit, 2009) and (Alemayehu *et al*., 2013). However, this finding agrees with the report of (Abebe *et al*., 2008) and the same observation was reported by (Lassen *et al*., 2009). In the present study, 48.7% and 46.6% of diarrheic and non-diarrheic animals were found to be positive to Eimeria, respectively. However, there were no

apparent clinical signs in most of the animals sampled for the study. The higher prevalence of Eimeria in diarrheic animals than in non-diarrheic animals suggests that diarrhea may also be caused by other microorganisms such as *Escherichia coli,* Salmonella, Cryptosporidium, rotavirus, etc. However, most obvious sign in affected cattle is diarrhoea containing mucus and blood, with frequent straining to pass faeces (DHHPS, 2011).

There was no significant association (P>0.05) recorded between body condition score and Eimeria infection in the current study. These indicate that body condition does not have influence on the occurrence of Eimeria infection. This is due to either the level of infection, sampled size or most of the affected animals harbor the disease without showing clinical signs (Fraser, 2006). The slight high prevalence can be explained by the fact that poor body condition animals harbor subclinical eimeriosis without showing clinical signs and the severity of the disease is low and might lead to lack of resistance to infection, decrease immune status of the animals and contribute for prevalence rate in poorly conditional animals (Abisola, 2004). In the current study, there was a higher prevalence rate in animals with poor body condition score than in animals with good and moderate body condition score. This might be also due to the weak immune status of the animals with poor body condition score. As a result, malnutrition and other parasitic infections result in immune compromised cattle. This condition produces a higher infection rate in poor state animals than in good-state animals (Radostits *et al*., 2007) There was no significant difference ($\chi 2$ = 0.8893, p =0.346) in the prevalence of eimeriosis and management systems. This result agrees with the report of (Abebe *et al*., 2008), (Alemayehu *et al.*, 2013) and (Temesgen, 2016). This similarity might be due to equal chance of accessing the oocysts when grazing from contaminated field. However, the current finding is in contrast with the previous report of (Abisola, 2004). This variation might be due to hygienic condition of the barn, nutritional status of the animals, contamination level of the feed, water, floor and treatment given to the animals. However, as the result shows it's more prevalent in intensive production system (50.4%) than the semi-intensive ones (45.3%); this was justified by (Radostitis *et al*., 1994) that eimeriosis in cattle is particularly a problem of confined animals kept under intensive husbandry practices.

In current study the overall prevalence of Cryptosporidium in dairy cattle was 10.8%. The current result is consistent with 10.6% in central parts of Ethiopia (Temesgen, 2004), 9.6% in Thailand (Jittapalapon *et al*., 2010) 11% of calves in Sweden (Bjorkman *et al*., 2003), 14% of cows in Denmark (Maddox-Hyttel *et al*., 2006) and 11.9% in USA in dairy cattle (Fayer *et al*., 2006). On the other hand, the current prevalence was lower than the previous reports of 24.0% in calves in Assela by (Berhanu *et al*., 2017), 27.8% in calves in Haramaya Ethiopia (Regassa *et al*., 2013b), 17.6% in dairy calves in central Ethiopia (Rahmeto *et al*., 2008), 17.9% in calves in France (Lefay *et al*., 2000), 19.2% in three cattle husbandry system in Zambia (Geurden *et al*., 2006), 23.4% in cattle in Nigeria (Ayinmode and Fagbemi, 2010), and 37.5% in cattle in Ogun state, Nigeria by (Akinkuotu *et al*., 2014). The lower prevalence of 2.3% in the country by (Adamu, 2010) was also reported. These differences in the prevalence among countries may be as a result of the difference in the stocking rate and husbandry system of livestock production system of the countries. It may also due to difference in climatic condition and seasonal variation during study. Besides these, variations could also be due to the difference in the susceptibility of the target population that related to age difference and breed of study animals and difficulty to diagnose Cryptosporidium oocyst (Geurden *et al*., 2006).

The results showed that the prevalence of Cryptosporidium infection in calves less than 6 month was the highest compared to those in yearling and adult cattle which supports the reports of (Lefay *et al*., 2000, Maddox-Hyttel *et al*., 2006 and (Ayinmode and Fagbemi, 2011) who observed that the prevalence of Cryptosporidium spp. in pre-weaned calves is usually high. Hence, in this study there was significance difference (P<0.05) between the age group and occurrence of Cryptosporidium. Analysis indicated that the occurrence of Cryptosporidium in calves less than 6 month was the highest with the percentage of 25% while in yearling and adult cattle, the percentage of occurrence was 12.8 % and 4.2%, respectively. So that the present study indicated that the younger animals were susceptible to infection with cryptosporidiosis as compared to adult animals. The higher prevalence in this age group can be related to the fact that these age groups are susceptible to the disease because of the immature immune system of the animal at this age. (Kvac *et al*., 2006) explained that the animal is becoming resistant with age due to the development immune system through time as the age of animals increase. However, the prevalence in adult animals indicated that there was subclinical infection without showing the clinical signs.

The results in this study showed no significant difference (P>0.05) in the rate of infection between female and male cattle as indicated by other studies (Ayinmode and Fagbemi, 2010; Mallinath *et al.,* 2009). The high rate of infection observed in male cattle is in tandem with reports of (Maikai *et al*.,

2011). The reason for this observation is not well known. But it may be due to husbandry practices given to male and female animal, size of sample, and difficulty diagnostic method etc.

Of the total 378 samples collected, 78 were diarrheic samples, and 300 were non-diarrheic ones. As shown in (Table 4), from 78 diarrheic samples, 20 (25.6) were positive for infection while out of 300 non-diarrheic samples, 21 (7%) were positive for Cryptosporidium oocysts. The results showed that there is statistically significant difference ($P<0.05$) between the presence of diarrhea and occurrence of infection. Comparable findings demonstrating association of Cryptosporidium with diarrhea had been reported (Del Coco *et al*., 2008; Diaz-Lee *et al*. 2011). In this case diarrhea occurs due to invasion and colonization of the intestinal epithelial surface by the parasite which results in loss of epithelial cells and microvillus brush border, increased epithelial permeability and osmotic diarrhea (Chen *et al*., 2002; Robinson *et al*., 2003).

The prevalence of cryptosporidiosis in dairy cattle with poor, medium and good body condition was 15.6%, 8.3% and 11.1%, respectively. There was no significant difference ($P>0.05$) in Cryptosporidium infection with in these three bodies condition score mean that there was equal chance of being infected with the infection. This finding was in contrary with (David *et al.,* 2000) and (Swai *et al*., 2007) in Tanzania observed that a poor body condition and diarrhea are the most prominent signs of cryptosporidiosis. On the other hand, other pathogens can result in poor body condition, immunocompromisation and increase new born calves susceptibility to cryptosporidium infection (Lefay *et al*., 2000). But higher prevalence was observed in poor body condition scores followed by good and medium body condition score. This difference might be due to subclinical infection and management practices in which poor body condition might be the result of poor husbandry system.

In this study out of 133 intensively managed cattle 24(12.8%) were found positive while out of 245 semi-intensively managed cattle, 17(9.8%) animals were found positive. The management system had no significant ($P = 0.373$) association on prevalence of Cryptosporidium infection. This finding was in agreement with previous observation of (Swai *et al.,* 2007) in Tanzania (56% in intensive, 28% in extensive and 16% in semi-intensive management). Previous studies by (Castro-Hermida *et al*., 2002 and Geurden *et al.,* 2006) was also explain that the higher stocking rate enhances the infection since infected calves produce large numbers of oocyst into confined dairy house ensuring a high environmental contamination.

5. Conclusion and Recommendations

In conclusion, this study provides proof of Eimeria and Cryptospordium infection in dairy farms in Holeta and its environs. This research has indicated that there were Cryptosporidium and Eimeria infection in cattle in Holeta, Ethiopia. From this study, it was concluded that infection of cattle in Holeta by these two protozoa seems very important. These parasites were observed in young calves and adults. However, the prevalence of Eimeria and Cryptosporidium was higher in young calves under 6 months of age than calves above 6 months of age and adult animals as a result of immature immune system. On the other hand, the excretion of Cryptosporidium oocyst was also significantly related to the presence of diarrhea. Concerning other risk factors, higher occurrence was observed in intensive management system than semi-intensive management system, in poor body condition, diarrheic and male animals. In addition, the study also indicated that adult animals disclosed subclinical infection with the presence of Eimeria and Cryptosporidium oocysts (Adult cows asymptomatic porters are important sources of infections). Eventhough most of the animals examined were found to be infected with Eimeria oocyts, clinical coccidiosis was not observed in infected animals. Thus, it can be emphasized that subclinical infection deserves attention as it may negatively influence productivity and causes economic losses. In general, the problem due to Cryptosporidium and Eimeria in the study area was given less attention because of its sub clinical nature of the disease especially in adult animals. So, the findings suggested that control and prevention measures must be taken in order to reduce the infection among cattle.

Therefore, based on the above conclusions, the following points were recommended:

- Good management practices and proper hygiene managements should be maintained. For instance, the dairy cattle owners should provide adequate colostrum to new born calves in order to control diarrhea caused by Cryptosporidioum, Eimeria and other pathogens.
- Moreover, avoiding of mixing of older animals with calves since older animals are the sole source of infection.
- Creation of awareness on the possible existence of Cryptosporidium infection is essential since it is zoonotic.
- Further study should be carried out in characterize the involving species of Eimeria and Cryptosporidium undertake epidemiological studies in more comprehensive manner.

References

1. Abebe R, Kumesa B, Wessene A. Epidemiology of Eimeria infections in calves in Addis Ababa and Debre Zeit Dairy Farms, Ethiopia. Intern J Appl Res Vet Med 2008; 6:24-30.
2. Abisola TO. Studies on Bovine coccidian. Apicomplexia: eimeriidae In: Parts of Plateau State, Nigeria (2004). Available at: *http://dspace.unijos.edu.ng/jspui/handle/123456789/129*. Accessed June 2, 2018.
3. Adamu H. The prevalence of intestinal parasites and molecular characterization of *Cryptosporidium* species in Ethiopia (2010). *http://www.13dl.handle.net/123456789/2436pdf*. Accessed June 2, 2018.
4. Akinkuotu OA, Fagbemi BO, Otesile EB, Dipeolu MA, Ayinmode AB. Cryptosporidium infection in cattle in Ogun state, Nigeria. Sokoto J of Vet Sci 2014; 12.
5. Alemayehu A, Nuru M, Belina T. Prevalence of bovine coccidian in Kombolcha district of South Wollo, Ethiopia. J Vet Med Anim Health 2013; 5(2):41-45.
6. Almeida VD, Magalhaes VC, Muniz-Neta ES, Munhoz AD. Frequency of species of the genus Eimeria in naturally infected cattle in Southern Bahia, Northeast Brazil. Brazilian J Vet Parasitol 2011; 20:78-81.
7. Ayinmode AB, Fagbemi BO. Prevalence of Cryptosporidium infection in cattle from southern Nigeria. Veterinarski Archiv 2010; 80:723-731.
8. Ayinmode AB, Fagbemi BO. Cross-reactivity of some Cryptosporidium species with *Cryptosporidium parvum* coproantigen in a commercial ELISA kit, Nigerian. Vet J 2011; 32:1-4.
9. Bahrami SE, Alborzi AR. Prevalence of subclinical coccidiosis in river buffalo calves of southwest of Iran. Acta Parasitologica 2013; 58:527-530.
10. Bandyopadhyay S, Mandal S, Datta K, Devi P, De S, Bera A, Bhattacharya D. Economic analysis of risk of gastrointestinal parasitic infection in cattle in North Eastern States. Trop Anim Health Prod 2010; 42:1481.
11. Bangoura B, Mundt H, Schmäschke R, Westphal B, Daugschies A. Prevalence of *Eimeria bovis* and *Eimeria zuernii* in German cattle herds and factors influencing oocyst excretion. Parasito Res 2012; 110:875-881.
12. Bekele M, Ferid D, Yeshitila A. Calf Coccidiosis in Selected Dairy Farms of Dire Dawa, Eastern Ethiopia. Global Vet 2012; 9:460-464.
13. Berhanu F, Lemma D, Eticha E, Abera B, Adem A. Prevalence and Risk Factors of Cryptosporidiosis in Dairy Calves in Asella Town, South Eastern, Ethiopia Acta Parasitologica Globalis 2017; 8(1):50-57.
14. Bjorkman C, Svensson C, Christensson B, De Verdier K. *Cryptosporidium parvum* and *Giardia intestinalis* in calf diarrhoea in Sweden. Acta Veterinaria Scandinavia 2003; 44:145-152.
15. Brook EJ, Hart CA, French NP, Christley RM. Prevalence and risk factors for Cryptosporidium spp. infection in young calves. Vet Parasitol 2008; 152:46-52.
16. Castro-Hermida J, González Losada Y, Ares Mazás E. Prevalence and risk factors involved in the spread of neonatal bovine Cryptosporidiosis in Galicia (NW Spain). Vet Parasito 2002; 106:1-10.
17. Chibunda RT, Muhairwa AP, Kambarage DM, Mtambo MM, Kusiluka LJ, Kazwala RR. Eimeriosis in dairy cattle farms in Morogoromunicipality of Tanzania. Prev Vet Med 1997; 31:191-197.
18. Cicek H, Sevimli F, Kozan E, Köse M, Eser M, Dogan N. Prevalence of coccidia in beef cattle in western Turkey. Parasito Res 2007; 101:1239-1243.
19. CSA. Federal Democratic Republic of Ethiopia: Central Statistical Agency Agricultural sample survey, report on Livestock and Livestock characteristics, Bulletin No.578. Addis Ababa, 2015; 12-15.
20. Das M, Deka D, Sarmah P, Islam S, Sarma S. Diversity of Eimeria spp. in dairy cattle of Guwahati, Assam India. Vet World 2015; 8:941-945.
21. Daugschies A, Najdrowski M. Eimeriosis in cattle: current understanding. J Vet Med B Infectious Diseases and Veterinary Public Health 2005; 52:417-427.
22. David L, Upton D, Owen R, Una M. Prevalence of Cryptosporidium infection in cattle from South Western Nigeria. Vet Arhiv 2000; 42.
23. Del Coco V, Co´rdoba M, Basualdo A. Cryptosporidium infection in calves from a rural area of Buenos Aires, Argentina. Vet Parasitol 2008; 158:31-35.
24. DHHPS Coccidiosis in Calves. *https://www.ed.ac.uk/files/imports/fileManager/Coccidiosis%20updated.pdf.* (2011). Accessed on June, 5 2018.
25. Díaz-Leea A, Mercado R, Onuoha E, Ozakif L, Munoz P, Munoz V, Martínez F, Fredes F. *Cryptosporidium parvum* in diarrheic calves detected by microscopy and identified by immunochromatographic and molecular methods. Vet Parasitol 2011; 176:139-144.

26. Dong H, Zhao Q, Han H, Jiang L, Zhu S, Li T, Kong C, Huang B. Prevalence of coccidian infection in dairy cattle in shanghais, China. J Parasitol 2012; 98:963-966.
27. Dorny P, Stoliar V, Charlier J, Meas S, Sorn S, Chea B, Holl D, Van Aken D Vercruvsse J. Infections with gastrointestinal nematodes, Fasciola and Paramphistomum in cattle in Cambodia and their association with morbidity parameters. Vet Parasitol 2011; 175:293-199.
28. Dryden M, Payne P, Ridley R, Smith V. Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. Vet Ther 2005; 6:15-2.
29. Edmonson A, Lean I, Weaver L, Farver T, Webster G. A Body Condition Scoring Chart for Holstein Dairy Cows. J Dairy Sci 1989; 72:3529.
30. Erastus K, Delia G, Cecilia M, Erastus M, Joseph M, Concepta N, Julie A, Alice N. Prevalence of Cryptosporidiosis in dairy cattle, cattle-keeping families, their non-cattle keeping neighbours and HIV-positive individuals in Dagoretti Division, Nairobi, Kenya. Trop Anim Health Prod 2012; 44:11-16.
31. Faber J, Kollmann D, Heise A, Bauer C, Failing K, Bürger H, Zahner H. Eimeria infections in cows in the periparturient phase and their calves: oocyst excretion and levels of specific serum and colostrum antibodies. Vet Parasitol 2002; 104:1-17.
32. Fayer R, Santin M, Trout JM, Greiner E. Prevalence of species and genotypes of Cryptosporidium found in 1-2 year old dairy cattle in the eastern United States. Vet. Parasitol 2006; 135:105-112.
33. Fraser CM. The Merck Veterinary Manual, A Hand Book of Diagnosis Therapy and Disease Prevention and Control for Veterinarians. In: Rahway 7th edition. Merck and Co., NIT, USA, 2006; 714-717.
34. Getahun T, Tamirat S, Aster Y, Melese E. Prevalence of gastrointestinal parasites in dry season on dairy cattle at Holeta Agricultural Research Center Dairy Farm, Ethiopia. J Vet Med 2017; 9:356-360.
35. Getrude S, George K, Charles M, John V, Shauna R, Jeff W, Fabienne U, Omwando M. Prevalence of Cryptosporidia, Eimeria, Giardia, and Strongyloides in pre-weaned calves on smallholder dairy farms in Mukurwe-ini district, Kenya. Veterinary World, EISSN. 2015; 2231-0916.
36. Geurden T, Goma F, Siwila J, Phiri I, Mwanza A, Gabriel S, Claerebout E, Vercruysse J. Prevalence and genotyping of Cryptosporidium in three cattle husbandry systems in Zambia. Vet Parasitol 2006; 138:217-222.
37. Gillhuber J, Rügamer D, Pfister K, Scheuerle M. Giardiosis and other enteropathogenic infections: A study on diarrhoeic calves in Southern Germany. BMc Res Notes 2014; 7:112-120.
38. Gormley F, Little C, Chalmers R, Rawal N, Adak G. Zoonotic Cryptosporidiosis from petting farms, England and Wales, 1992-2009. Emerg Infect Dis 2011; 17:151-152.
39. Heidari H, Gharekhani J. Detection of Eimeria species in Iranian native cattle. Int J Adv Res 2014; 2:731-734.
40. Heidari H, Sadeghi-Dehkordi Z, Moayedi R, Gharekhani J. Occurrence and diversity of Eimeria species in cattle in Hamedan province, Iran. Vet Med 2014; 59:271-275.
41. Huang J, Yue D, Qi M, Wang R, Zhao J, Li J, Shi K, Wang M, Zhang L. Prevalence and molecular characterization of Cryptosporidium spp. and Giardia duodenalis in dairy cattle in Ningxia, northwestern China. Vet Res 2014; 10:292.
42. Hussin AG. Prevalence and Associated Risk Factors of Eimeria spp. in cattle of Baghdad, Iraq. J Appl Anim Sci 2016; 9:37-44.
43. Ibrahim D. Prevalence and associated risk factors of calf coccidiosis in and around Asela Town, Southeast Ethiopia. 2016; 6.
44. Ikimap. *http://www.ikimap.com/map/administrative-area-walmara-ethiopia*. (2018). Accessed on June, 2 2018.
45. Izadi M, Jonaidi-Jafari N, Saburi A, Eyni H, Rezaiemanesh M, Ranjbar R. Cryptosporidiosis in Iranian farm workers and their household members: a hypothesis about possible zoonotic transmission. J Trop Med 2014; 7.
46. Jatapalapon S, Pinyopanuwat C, Siripanth C. Prevalence of Cryptospordium among dairy cows in Thailand department of parasitology, Faculty of veterinary medicine kastesant university, Bangkok 2006; 1081:328-335.
47. Johannes K. Parasitic infections of domestic animals. A Diagnostic Manual, 1996; 24-27.
48. Kaupke A, Michalski MM, Rzeżutka A. Diversity of Cryptosporidium species occurring in sheep and goat breeds reared in Poland. Parasitol Res 2017; DOI 10.1007/s00436-016-5360-3.
49. Korich D, Marshall M, Smith H, O'grady J, Bukhari Z, Fricker C, Rosen J, Clancy J. Inter-laboratory comparison of the CD-1 neonatal mouse logistic dose-response model for *Cryptosporidium parvum* oocysts. J Eukaryot Microbiol 2000; 47:294-298.

50. Kvac M, Kouba M, Vitovec J. Age-related and housing dependence of Cryptosporidium infection of calves from dairy and beef herds in South Bohemia, Czech Republic. Vet Parasitol 2006; 137:202-209.
51. Lassen B, Viltrop A, Raaperi K, Jarvis T. Eimeria and Cryptosporidium in Estonian dairy farms in regard to age, species, and diarrhea. Vet Parasitol 2009; 166:212-219.
52. Lefay D, Naciri M, Poirier P, Chermett R. Prevalence of Cryptosporidium infection in calves in France. Vet Parasitol 2000; 89:1-9.
53. Lendner M, Daugschies A. Cryptosporidium infections: molecular advances. Parasitol 2014; 141:1511-1532.
54. Maddox-Hyttel C, Langkjaer R, Enemark H, Vigre H. Cryptosporidium and Giardia in different age groups of Danish cattle and pigs-Occurrence and management associated risk factors. Vet Parasitol 2006; 14:48-59.
55. Maikai B, Umoh J, Kwaga J, Lawal I, Maikai V, Camae V, Xiao L. Molecular characterization of Cryptosporidium spp. in native breeds of cattle in Kaduna State, Nigeria. Vet Parasitol 2011:178:241-245.
56. Mallinath RH, Chikkachowdappa PG, Gowda AK, D'souza PE. Studies on the prevalence of Cryptosporidiosis in bovines in organized dairy farms in and around Bangalore, South India. Vet arhiv 2009; 79:461-470.
57. Matjila PT, Penzhorn BL. Occurrence and diversity of bovine coccidia at three localities in South Africa. Vet Parasitol 2002; 104:93-102.
58. Nagwai II, Faragaila M, Moghazy E, Soad EH. Diagnosis of Eimeriosis in cattle by ELISA using partially purified antigen. World Appl Sci J 2011; 12:33-88.
59. Nisar-Khan M, Rehman T, Sajid M Abbas R, Zaman M, Sikandar A, Riaz M. Determinants influencing prevalence of coccidiosis in Pakistani buffaloes. Pakistan Vet J 2013; 33:287-290.
60. Ortegon H. Guidelines for age verification in cattle. Alberta agriculture and rural development, 2013; 4-8.
61. Pandit BA. Prevalence of Coccidiosis in Cattle in Kashmir valley. Vet Scan 2009; 4:16-20.
62. Radostitis O, Blood D, Gay C. Veterinary Medicine. 8th edition. Bailliere Tindall, London, 1994; 1201-1203.
63. Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Diseases associated with protozoa. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Goats, Pigs and Horses. 10th edition. Philadelphia, Pennsylvania: Saunders Elsevier. 2007; 1498-1515.
64. Rahmeto A, Abebe W, Bersissa K. An epidemiological study of Cryptosporidium infection in dairy calves on selected dairy farms of central Ethiopia. Revue Med Vet 2008; 159:107-111.
65. Regassa A, Gizaw O, Abunna F, Abebe R, Beyene D, Megersa B, Debela E, Asmare K, Skierve *E*. Cryptosporidium in Calves, Lambs and Kids at Haramaya, eastern Ethiopia. Ethiop Vet J 2013; 17:81-94.
66. Rehman T, Khan M, Sajid M, Abbas R, Arshad M, Iqbal Z, Iqbal A. Epidemiology of Eimeria and associated risk factors in cattle of District Toba Tek Singh. Pak. Parasitol Res 2011; 108:1171-1177.
67. Robinson P, Okhuysen P, Chappell C, Weinstock J, Lewis D, Actor J, White A. Substance expression correlates with severity of diarrhoea in Cryptosporidiosis. J Infec Dis 2003; 188:290-296.
68. Rodriguez-Vivas RI, Dominguez-Alpizar JL, Torres-Acosta JF. Epidemiological factors associated to bovine coccidiosis in calves (Bosindicus) in a sub humid tropical climate. Rev Biomed 1996; **7**:211-218.
69. Svensson C. Excretion of *Eimeria alabamensis* oocysts in grazing calves and young stock. Journal of Veterinary Medicine Infectious Diseases and Veterinary Public Health 2000; 47:105-110.
70. Swai ES, French NP, Karimuribo ED, Fitzpatrick JL, Bryant MJ, Kambarage DM, Ogden NH. Prevalence and determinants of Cryptosporidium Species infection in smallholder dairy cattle in Iringa and Tanga Regions of Tanzania. Onderst J Vet Res 2007; 74:23-29.
71. Taylor M. Protozoal disease in cattle and sheep. In: Practice 2000; 22:604-617.
72. Taylor MA, Coop RL, Wall LR. Veterinary Parasitology. 3rd edition. Blackwell Publishing, 2007; 627-628.
73. Temesgen GK. Epidemiological studies on calf coccidiosis in dairy farms in South Wollo Zone Amhara Region, Ethiopia. J Vet Sci Technol 2016; 7:2.
74. Temesgen W. Calf morbidity and mortality in dairy farms in Debre-Zeit and its environs. MSc Thesis, FVM, AAU, Debre-Zeit, Ethiopia, 2004.
75. Thrusfield M. Veterinary Epidemiology. 3rd edition. Blackwell Science Ltd., Oxford, UK, 2007; 233-261.
76. Tizard IR. An Introduction. 8th edition. Philadelphia PA: Saunders Company. Veterinary Immunology; 2009; 224-238.

77. Veses O. SFD Report - Holleta, Ethiopia - SFD Promotion Initiative. University of Leeds (UoL), 2016.
78. von Samson-Himmelstjerna G, Epe C, Wirtherle N, von der Heyden V, Welz C, Radeloff I, Krieger K. Clinical and epidemiological characteristics of Eimeria infections in first year grazing cattle. Vet Parasitol 2006; 136:215-221.
79. WoWAHA: Wolmera Wereda Animal Health Agency. Statistical Abstract. Wolmera, Finfine Liyu Zuria Zone, Oromia, Ethiopia, 2015.
80. Wymann MN. PhD Thesis. Germany: Calf mortality and parasitism in peri-urban livestock production in Mali. Dissertation University of Basel. http://www.edoc.unibas.ch/281/1/DissB_7204.pdf. (2005). Accessed on June 3, 2018.
81. Yadessa T, Awash H, Gurmu G. Prevalence of calves Coccidiosis in Jimma town dairy farms, South Western Ethiopia. Sci J Zoo 2014; 3:36-44.
82. Yu SK, Gao M, Huang N, Jia YQ, Lin Q. Prevalence of coccidial infection in cattle in Shaanxi province, Northwestern China. J Anim Vet Adv 2011; 10:2716-2719.
83. Zegeye Y. Imperative and challenges of dairy production, processing and marketing in Ethiopia. In: Jobre Y and Gebru G (eds), Challenges and opportunities of livestock marketing in Ethiopia. Proceedings of the 10th annual conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia. 2003; 61-67

7. Annexes

Annex-1: Floatation techniques preparation and procedure.

❖ Preparation of floatation fluid (sheathers sucrose soloution≈1.27 sp. gr.)
✓ 454 g table sugar
✓ 355 ml tap water
✓ 6 ml full-strength (37%) formaldehyde as a preservative.
✓ Water was heated to get sugar into solution (Dryden *et al*., 2006)
❖ Principle
➢ Based on the separating of eggs/oocysts from fecal materials flotation technique identify nematode, cystode and coccidian oocysts in the feces.
➢ It is by concentrating them by means of a floatation fluid with an appropriate specific gravity.
❖ Materials**:**

Microscopic slides
Measuring cylinder
Pistil and mortem
Disposable plastic glove
Cover slip
Tea spoon
Balance
Stick
Gown
Applicator
Microscope
Table sugar
Stove
A tea strainer
Beaker
Formalin (10%)
Test tube
Fecal floatation solution (sheather's solution)
Tap water Test tube rack

❖ Procedure of simple test tube floatation technique
✓ Three grams of feces were weighted from each animal and mixed with 42ml of sugar solution.
✓ Then it was poured through tea strainer into a beaker and then the solution was added into 15ml test tube.
✓ The test tube was gently top up with more floatation fluid with, leaving a convex meniscus at the top of the tube and then covered with cover slip on the tube and kept at standing for 20 minutes.
✓ Finally, Then the cover slip was carefully lift off from the tube vertically, together with the drop of fluid adhering to it, and placed on a microscope slide labeled with the animal name or number.
✓ The entire cover slip was examined at 10X to identify oocysts and results were recorded. The oocycts appeared as circular and ellipsoidal in shape with microphyle at one end (Dryden *et al*., 2006).

Annex-2: Modified Ziehl Neelsen acid fast staining

Material and reagents: 0.3% Carbol Fuchsin
25% Sulphuric acid
0.3% methylene blue
Methanol alcohol 95%
Microscopic slide
Rack
Pipette
Microscope
Tap water

❖ Preparation
✓ Basic fuchsin (4.0 g) -phenol (fresh, 8 ml)
✓ Mix fuchsin and phenol into a slurry
✓ Add 95% ethanol (20 ml) and mix
✓ Add de-ionized water (100 ml) and mix well and filter prior to use

- ❖ Procedure
- ✓ The air dried smear was fixed with concentrated methanol for 3 minutes
- ✓ A few drops of carbol fuschin was added to the smear for 5 minutes
- ✓ The slide was rinsed thoroughly in tap water
- ✓ Decolorized in acid alcohol (25% $H2S0_4$) for 30 second
- ✓ Rinsed in tap water
- ✓ Counterstained with methylene blue for 30 seconds
- ✓ Rinsed in tap water
- ✓ The slide was dried
- ✓ The slide was examined using 40x and 100x (under oil immersion) objective lenses
- ✓ The sporozoites appeared as bright red granules (Korich *et al*., 2000)

Annex-3: Body condition scoring system

- ❖ **Poor**: when there is little evidence of fat deposition but some muscling in the hindquarters and the spinous processes feel sharp to the touch and are easily seen with space between them.
- ❖ **Moderate**: when the spinous process can be felt with very firm pressure and they were round rather than sharp and there is evidence of moderate fat cover.
- ❖ **Good**: when the *Tail head* had fat cover over whole area and skin smooth but pelvis can be felt and end of horizontal process can only be felt with pressure; only slight depression in loin (Edmonson *et al*., 1989).

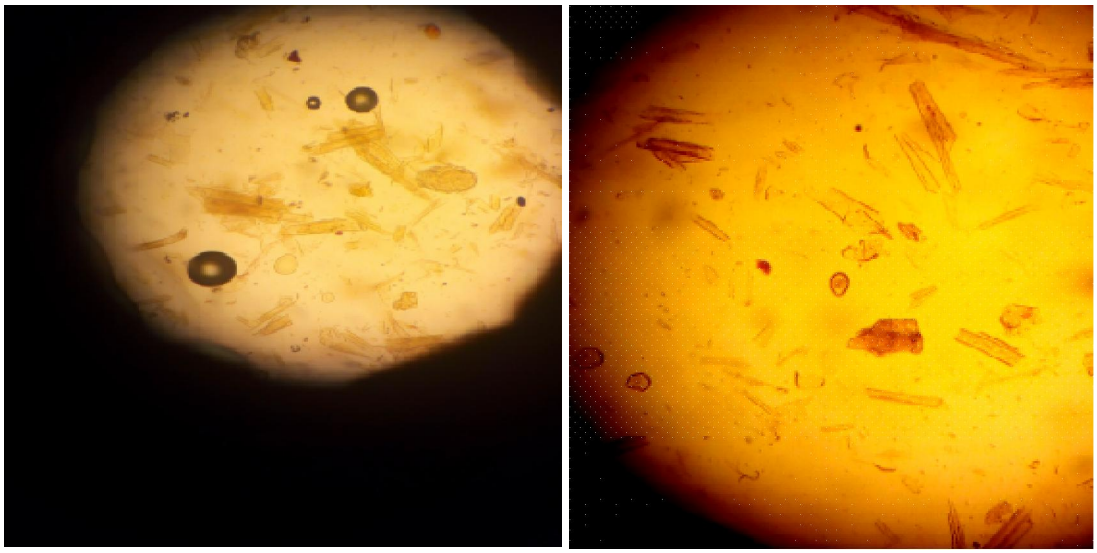
Annex-4: Age determination

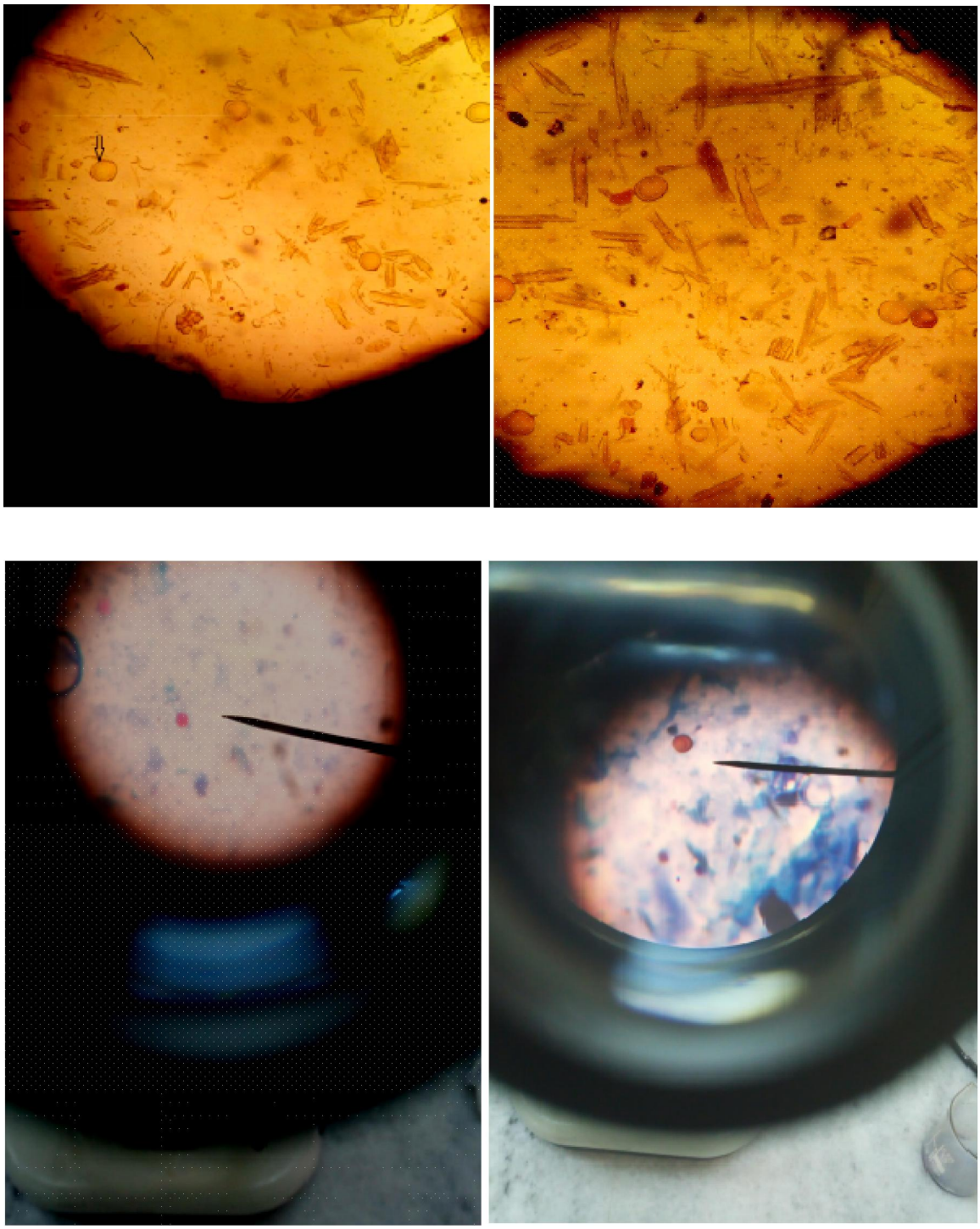
- ✓ At birth to 1month, two or more of the temporary incisor teeth present. Within first month, entire 8 temporary incisors appear.
- ✓ Age 10 months- a calf mouth showing external face of the incisor teeth.
- ✓ 12 months - All the calf teeth are in place.
- ✓ 15 months - Centre permanent incisors appear.
- ✓ 18 months - Centre permanent incisors showing some wear.
- ✓ The central pair of temporary incisors are replaced by permanent ones which attains full growth by 2 years.
- ✓ The third permanent incisor erupts at around 30 months of age. The fourth permanent incisors erupt after 30 months. The second pair of incisors is fully developed at 3 years.
- ✓ By the 4-5 years the animal has a full set of permanent incisors. By the sixth year, the central incisor shows wear and leveled top (Ortegon, 2013).

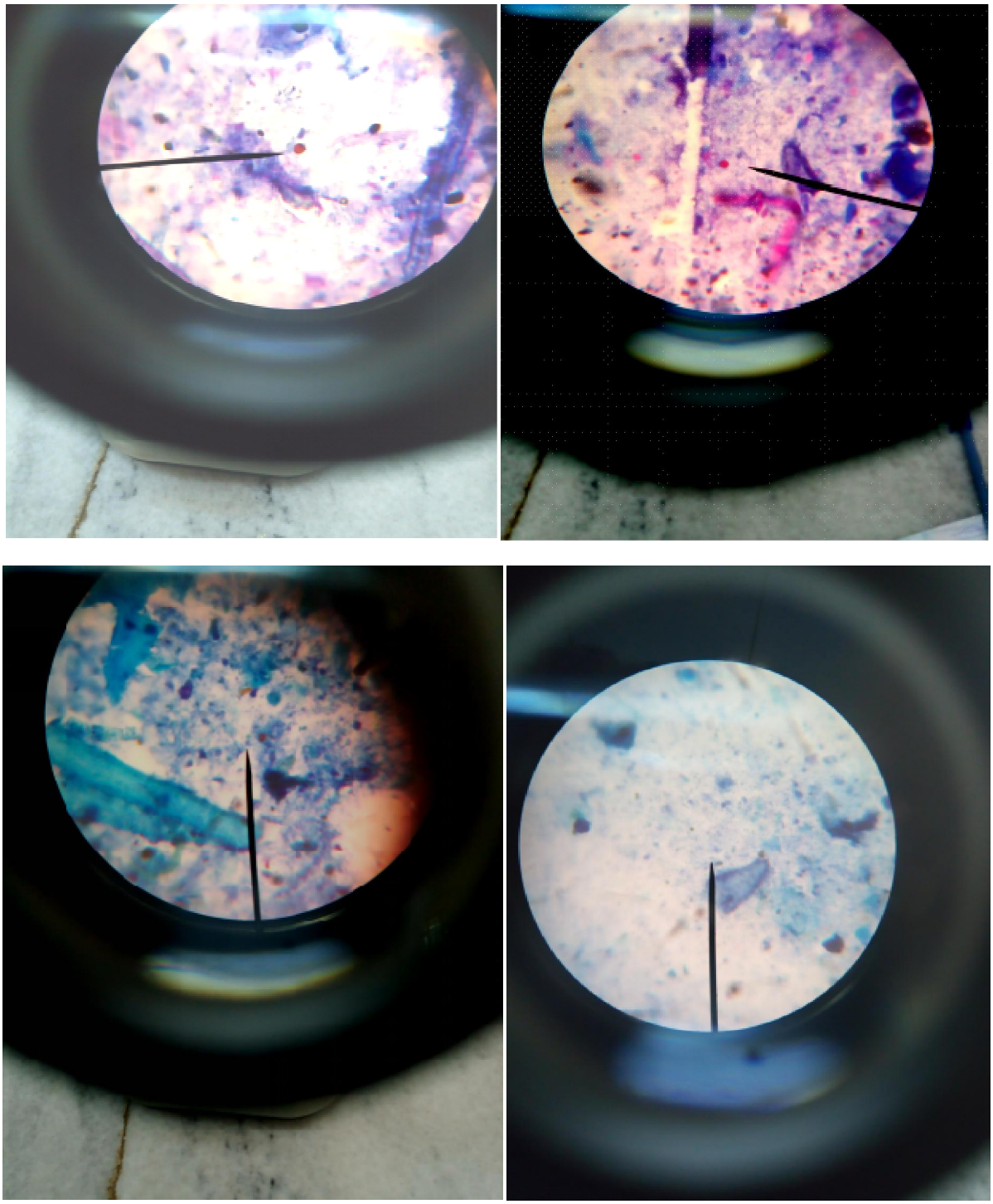
Annex-5: Sample collection format

Date	Code	Farm name	Sex	Age	Management	Fecal Consistency	Body condition	Eimeria	Crypto	Result

Annex-6: Picture of examined Eimeria and Cryptosporidium oocyst under light microscope.







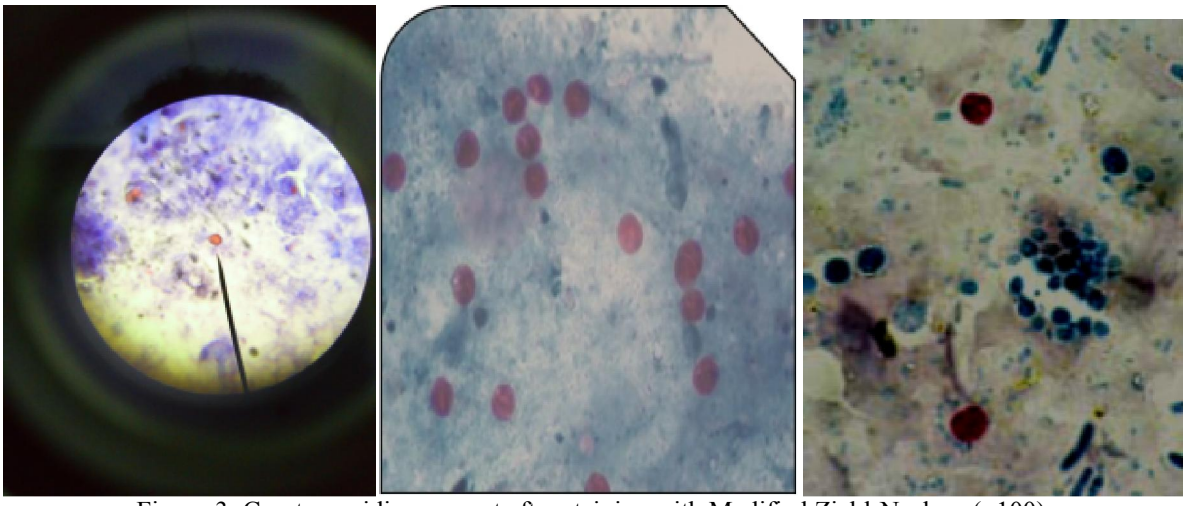


Figure-3: Cryptosporidium oocyst after staining with Modified Ziehl-Neelsen (×100)

8/20/2020