ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

AN EPIDEMIOLOGICAL STUDY ON MAJOR PROTOZOAL CAUSES OF CALF DIARRHEA ON SELECTED DAIRY FARMS OF CENTRAL ETHIOPIA

BY
RAHMETO ABEBE BESHIR

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Debre Zeit, Ethiopia

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical Veterinary Epidemiology

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AAU	Addis Ababa University
AIDS	Acquired immune deficiency syndrome
ANOVA	Analysis of variance
CI	Confidence interval
CSA	Central Statistics Authority
DNA	Deoxyribonucleic acid
DHM	Dairy herd management
ELISA	Enzyme-linked immunosorbent assay
FAT	Fluorescent antibody test
FVM	Faculty of Veterinary Medicine
IF	Immunofluorescence
IBR	Infectious bovine rhinotracheitis
ISU	Iowa State University
LAT	Latex agglutination test
LAV	Large animal veterinarian
Mabs	Monoclonal antibodies
NMA	National Metrology Agency
NA	North America
OPG	Oocysts per gram of feces
OR	Odds Ratio
PCR	Polymerase chain reaction
RPH	Reverse passive haemaglutination
SPSS	Statistical packages for the social science
Spp	Species
$\chi 2$	Chi-square

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ABSTRACT

A cross-sectional study was undertaken from September 2004 to March 2005 on selected 40 dairy farms located in Central Ethiopia to determine the prevalence and species of the major protozoal causes of calf diarrhea, and the factors associated with their occurrence. Fecal samples were collected once from a total of 580 calves 0-345 days old and examined for the oocysts of *Cryptosporidium* and *Eimeria spp*. by centrifugal fecal flotation technique using concentrated sucrose solution. Accordingly, an overall point prevalence of 17.6% and 68.1% was recorded for *Cryptosporidium* and *Eimeria spp*., respectively. Of the 40 dairies sampled, 26 (65%) had one or more calves shedding *Cryptosporidium* oocysts whereas all of the farms had calves shedding *Eimeria* oocysts. In this study the species of *Cryptosporidium* circulating in the farms was presumed to be *Cryptosporidium andersoni* (formerly known as *Cryptosporidium muris*) based on morphology of the oocysts and certain epidemiological features of the parasite. With regard to the species of *Eimeria*, a total of 11 species were identified namely *Eimeria bovis*, *Eimeria zuernii*, *Eimeria auburnensis*, *Eimeria canadensis*, *Eimeria ellipsoidalis*, *Eimeria subspherica*, *Eimeria cylindrica, Eimeria alabamensis*, *Eimeria wyomingensis*, *Eimeria bukidnonensis*, and *Eimeria brasilensis* in descending order of their relative prevalence. Neither *Cryptosporidium* nor *Eimeria spp*. were found to be statistically associated with diarrhea (P>0.05). Both parasites were detected in a wide age range of calves: *Cryptosporidium* in 21-345-day old calves and *Eimeria spp*. in 15-345-day old calves. Among the risk factors studied, the frequency of cleaning the calf rearing houses was the most important factor associated with the likelihood of infection with *Cryptosporidium spp*. whereas geographical zone where samples were taken from and age of the calves were the most important factors associated with the likelihood of infection with *Eimeria spp*. The mean and maximum *Eimeria* oocysts per gram of feces (OPG) determined by using McMaster technique was 5109 and 267,000, respectively. There was a strong association between OPG and age of the calves (P<0.001) and consistency of the feces (P<0.001), the mean OPG was significantly higher in younger and diarrheic calves.

Keywords: Calf diarrhea; *Cryptosporidium*; *Eimeria*; Prevalence; Risk factors; Species

1. INTRODUCTION

The future of any dairy production depends, among other things on successful program of raising calves and heifers for replacement (Bath *et al*., 1985). On the other hand, the health and management of replacement animals are important components of total herd profitability. The productivity of the herd can be negatively impacted by impaired growth of calves, decreased milk production of animals that experienced chronic illness as baby calves, spread of infectious diseases from calves to adult cows, increased veterinary costs and the limited opportunity for genetic selection due to high mortality of replacement animals. Amongst all animals present on a dairy farm, the highest morbidity rates generally occur in baby calves prior to weaning (McGuirk and Ruegg, 2004).

The two most important disease problems in the young calf are diarrhea and pneumonia (Heinrichs and Radostits, 2001). Diarrhea is the most serious and common health problem particularly in neonatal calf that is responsible for the greatest economic losses in this age group for both dairy and beef calves (Heath, 1992), and as reported by Heinrichs and Radostits (2001), acute diarrhea accounts for approximately 75% of the mortality losses of dairy calves less than 3 weeks age.

The diarrheal syndrome has a complex etiopathogenesis, because various infectious agents, either alone or in combination, may be associated with field outbreaks. In addition, environmental, management, and nutritional factors influence the severity and out come of the disease (Reynolds *et al*., 1986). According to Heinrichs and Radostits (2001), the most important pathogens associated with calf diarrhea worldwide are ETEC, rotavirus, coronavirus, Salmonella species, and protozoan parasites *Cryptosporidium* and *Eimeria* species. The latter two enteropathogens are the subject of this paper.

Cryptosporidiosis is a widespread parasitic disease caused by apicomplexan parasites of the genus *Cryptosporidium* (Tyzzer, 1907), which develop and multiply in the epithelial cells of the gastrointestinal or respiratory tracts of a wide range of mammals, birds, reptiles, and fish.

Infected individuals show a wide spectrum of clinical presentations, but the pathogenecity of *Cryptosporidium* varies with the species of parasites involved and the type, age, and immune status of the host (Xiao *et al*., 2004). Based on transmission experiments *C. parvum*, which is one of the two important species of *Cryptosporidium* in cattle, was shown to lack host specificity and therefore a potential zoonosis. In cattle the clinical picture, which emerges from field reports, is one of mild to severe diarrhea occurring in calves between 1 and 4 weeks, with high morbidity and low mortality (de Graaf *et al*., 1999).

For a long time *Cryptosporidium* has been regarded as an opportunistic organism occurring in conjunction with other enteric pathogens such as rotavirus, coronavirus and ETEC in severely diseased animals (Reynolds *et al*., 1986; Snodgrass *et al.,* 1986: Moore and Zeman, 1991; de la Fuente *et al.,* 1999). However, several experimental and field studies have demonstrated that it may act alone to cause severe diarrhea (Moore and Zeman, 1991; de la Fuente *et al*., 1999; Scott *et al.,* 1995) and death (Moore and Zeman, 1991; Sanford and Josephson, 1982) in neonatal calves even in the absence of other enteropathogens. In addition to its impact on the health and productivity of calves, the fact that *Cryptosporidium* was found to infect humans, and could cause a life-threatening disease in immunodeficient people, especially AIDS patients (Martins and Guerrant, 1995), as well as the association of *Cryptosporidium* with water-borne related human outbreaks of diarrhea (Mackenzie *et al*., 1994), has certainly given the parasite a more widespread recognition.

Coccidiosis is also another important protozoal disease caused by apicomplexan parasites of the genus *Eimeria*. The parasite infects the intestinal tract and causes mild to severe diarrhea, most frequently in calves 6 to 12 months (Kennedy, 2001) although occasionally occurs in yearlings and adults (Soulsby, 1982; Urquhart *et al*., 1996). It is one of the most economically important parasitic diseases of cattle (Dedrickson, 2002; Kirckpatrick and Selk, 2003), which is actually characterized by two different syndromes: subclinical and clinical (Richey, 2004; Dedrickson, 2002). Compared to clinical coccidiosis, nonapparent or subclinical coccidiosis is more important and may account for over 95% of all the losses associated with coccidiosis (Dedrickson, 2002). These silent, subclinical infections cause substantial economic losses due to poorer feed

efficiency, slow weight gain, weight loss, longer heifer development periods and increased susceptibility to other disease causing agents (DHM, 1998).

In Ethiopia, although diarrhea is an important cause of calf morbidity and mortality, studies done to quantify the magnitude of the problem and to determine the underlying causes are scant and scarce. The available scant data also refer to only bacterial and viral causes and no attention has been given to the role of cryptosporidiosis and coccidiosis as causes of disease and production losses in cattle, although these diseases are of foremost importance in most parts of the world in view of their economic repercussions not only due to the high mortality they sometimes cause, but also because of failure of young stock to grow and gain weight to their full potential, reduction in body weight, and medication and labor costs.

Therefore, taking these facts in to account a cross-sectional study, the first of its kind in Ethiopia, was undertaken in selected dairy farms in central Ethiopia with the following objectives:

- To determine the prevalence and the species of the major protozoan agents causing calf diarrhea with especial emphasis on *Cryptosporidium* and *Eimeria* species.

- To identify the potential risk factors associated with occurrence of cryptosporidiosis and coccidiosis in the farms.

- To recommend appropriate prevention and control measures based on the findings of the study.

2. LITERATURE REVIEW

2.1 Historical background

The first description of *Cryptosporidium* was made by Tyzzer (1907), when he isolated the type species, which he named *Cryptosporidium muris*, from the gastric glands of laboratory mice. He later published a more complete description of the life cycle in 1910 and both asexual and sexual developmental stages were described to culminate in the formation of unique spores (or oocysts) containing 4 sporozoites not enclosed within secondary spores (sporocysts). Tyzzer (1912) found a second isolate, which he named *C. parvum*, in the small intestine of the same species of laboratory mice but they were smaller than *C. muris* and their development was confined to the small intestinal epithelium.

Infection by *Cryptosporidium* species in cattle was first reported by Panciera *et al.* (1971) in the intestines of diarrheic calves. However, due to its association with other bacterial and viral enteropathogens, its role as a primary pathogen was not established until the 1980's when Tzipori *et al.* (1980) attributed an outbreak of neonatal diarrhea in calves to parasitization by *Cryptosporidium* alone. In the following years methods to free the infective oocysts from other contaminating pathogens become available, which permitted the experimental demonstration that *Cryptosporidium* was capable of causing clinical diarrhea in calves (Tzipori *et al.*, 1983). At present, *Cryptosporidium* infection is a well-recognized cause of diarrhea in immunologically healthy and immunocompromised humans and animals of agricultural interest throughout the world (Abrahamsen, 1998). Only *C. parvum* is known to be associated with diarrhea in neonatal ruminants (de Graaf *et al*., 1999).

Coccidiosis as a distinct clinical disease of cattle was first described by F. A. Zurn in 1878 (cited by Ernst and Benz, 1981). Zurn observed coccidian intracellular stages in sections of intestine from a calf that had died of enteritis and sent from Switzerland. The disease encountered was given the name rote Ruhr ("red dysentery"). Unfortunately, Zurn did not give a detailed description of the parasitic stages he saw and hence present – day investigators cannot distinguish

that species from the other species, which are now recognized from cattle. *Eimeria zuernii* is apparently the most common cause of rote Ruhr in Switzerland and may have been the species that Zurn saw (Ernst and Benz, 1981).

Bovine coccidiosis has been given several common or colloquial names in addition to rote Ruhr; these include scours, bloody scours, hemorrhagic enteritis, dysentery and bloody diarrhea. The most precise name for this disease is bovine eimeriosis, since all coccidial organisms of cattle that cause coccidiosis belong to the genus *Eimeria*. However, bovine coccidiosis is the most commonly used name and is preferred by most investigators (Ernst and Benz, 1981).

2.2 Etiology

Cryptosporidiosis is caused by obligate, intracellular, protozoan parasites that belong to the genus *Cryptosporidium* (Tyzzer; 1907; Fayer *et al.,* 2000; Upton, 2003). Named species of *Cryptosporidium* that are currently considered valid species now include *C. andersoni* (cattle), *C. baileyi* (chicken and some other birds), *C. canis* (dogs), *C. felis* (cats), *C. galli* (birds), *C. hominis* (humans), *C. meleagridis* (birds and humans), *C. molnari* (fish), *C. muris* (rodents and some other mammals), *C. parvum* (ruminants and humans), *C. wrairi* (guinea pigs), *C. saurophilum* (lizards and snakes), and *C. serpentis* (snakes and lizards) (Xiao *et al*., 2004). However only two of these protozoan parasites: *Cryptosporidium parvum* and *Cryptosporidium andersoni* (formerly known as *C. muris;* Lindsay *et al*., 2000*)* are important in cattle (Anderson, 1998; de Graaf *et al*., 1999; Upton and Current, 1985; Lindsay *et al*., 2000).

Cryptosporidium muris was thought to be a gastric pathogen in rodents and cattle, and is recognized as a valid species. However, recent molecular analyses have demonstrated that *C. muris* in cattle is genetically distinct from *C. muris* in rodents, despite their morphological similarity. It has also been reported that *C. muris* from cattle were unable to infect laboratory rodents that were immunocompetent or immunocompromised, although *C. muris* from rodents were able to infect these laboratory rodents (Lindsay *et al*., 2000). Based on these findings, *C. muris* in cattle have been proposed to be a new species and now renamed, *C. andersoni* (Lindsay

et al. 2000), which has been recognized as valid (Xiao *et al*., 2004). It was named after Bruce Anderson, University of Idaho, and the original finder of the parasite.

Bovine coccidiosis is also caused by intracellular protozoan parasites chiefly of genus *Eimeria* (Ernst and Benz, 1986; Ferguson, 1996; Adams and Perth, 2004). The number of species of *Eimeria* that occur in cattle is open to question, but most reviewers accept 13 (Ernst and Benz, 1986; Levine, 1985; Urquhart *et al.*, 1996; Adams and Perth, 2004) to 21(Kaufmann, 1996; Ferguson, 1996). However, only *Eimeria bovis* and *Eimeria zuernii* are highly pathogenic and regularly associated with clinical infections in the field (Ernst and Benz, 1986; Ferguson, 1996; Urquhart *et al.,* 1996; LAV, 1996; Kaufmann, 1996; Adams and Perth, 2004). Experimentally other species have been shown to be mildly, or moderately pathogenic (Aiello and Mays, 1998).

2.3 Parasite morphology and site of infection

The two species of *Cryptosporidium* recognized in cattle are well differentiated on the basis of oocyst morphology and site of infection (O'Donoghue, 1995; Anderson, 1998). The oocysts of *C. parvum* are nearly spherical in shape and have an average size of 5.0 µm x 4.5 µm (Upton and Current, 1985; Fayer and Ungar, 1986). As to the site of parasitism, *C. parvum* infects the epithelial cells of the distal small intestine mainly ileum. *C. andersoni* infects the abomasum of cattle and the oocysts are ellipsoid that measure 7.4 x 5.5µm (Lindsay *et al*., 2000). In host tissue *Cryptosporidia* develop intracellularly but extracytoplasmic, which may contribute to the marked resistance of the organisms to treatment (Eisen, 2004). The *Eimeria* species known in cattle vary considerably in their morphology and a detailed description on their morphology and other features is given in Annex 1. The development of *Eimeria* species inside the host takes place intracellularly in the epithelial cells of terminal ileum, caecum and colon (Kaufmann, 1996; Radostits and Stockdale, 1980; Bowman, 1999).

2.4 Classification /Taxonomy

According to the current taxonomic classification, the causative agent of cryptosporidiosis belongs to the Phylum Apicomplexa, Class Conoidasida, Subclass Coccidiasina, Order Eucoccidiorida, Suborder Eimeriorina, Family Cryptosporidiidae and Genus *Cryptosporidium* (Upton, 2003). The same classification also holds true for *Eimeria* except that they belong to a different Family, Eimeriidae and Genus *Eimeria* (Urquhart *et al*., 1996)

2.5 Epidemiology

2.5.1 Geographical distribution and prevalence

Bovine cryptosporidiosis has worldwide distribution (Aiello and Mays, 1998; ISU, 2003). Since the first report of its presence in cattle, the disease has been the object of many prevalence studies world wide and has been documented in people and animals in 95 countries (Fayer *et al.*, 1998) ranging in location from tropical to temperate zones throughout the world (O'Donoghue, 1995). Most infections have been attributed to *C. parvum* associated with clinical disease (O'Donoghue, 1995). Most of the published studies were from North America, Europe, and Japan and little is known on the prevalence of bovine cryptosporidiosis in African countries. The reported prevalences vary according to the species involved, the age group affected and the study design used (Table 1).

In cross-sectional studies involving single samplings the reported point prevalence ranges from 0.5% to 65.8%, whereas in longitudinal studies period prevalences of 5% to 100% have been reported based on repeated samplings. Prevalences reported from different countries in the world are presented in Table 1. As indicated in the table most of the reports are that of *C. parvum* mainly in preweaned or newborn calves. *C. andersoni (C. muris)* is much less prevalent and was only found in weaned calves or adult cattle, and infections have not been associated with any sign of acute disease (Anderson, 1989; O'Donoghue, 1995; Bukhari and smith, 1996; de J. Pena *et al.*, 1997; Wade *et al.*, 2000).

Table 1. Prevalence rate of *Cryptosporidium* spp. in different countries of the world

Country	**Reported prevalence**		**Age reported**	**Species**	**Source**
	Type	**Rate (%)**			
USA	Point prevalence	5.6	1-11months	*C. parvum*	Atwill *et al.* (1999)
USA	Point prevalence	0.9	0-30 days	*C. parvum*	Wade *et al*. (2000)
USA	Point prevalence	1.1	51days to adults	*C. muris**	Wade *et al.* (2000)
USA	Period prevalence	92	7-21 days	*C. parvum*	Atwill *et al*. (1998)
USA	Point prevalence	4.7-31	Not reported	*C. muris**	Anderson (1991)
USA	Period prevalence	100	0-20 weeks	*C. parvum*	Xiao and Herd (1994)
USA	Period prevalence	100	1-30 days	*C. parvum*	McCluskey *et al*. (1995)
USA	Point prevalence	22.4	0-505 days	Not reported	Garber *et al*. (1994)
USA	Point prevalence	0.5-3.3	Adult cattle	*C. muris**	Anderson (1987, 1989)
USA	Point prevalence	1.03	Calves	*C. muris**	Anderson (1989)
Spain	Point prevalence	47.9	0-3weeks	*C. parvum*	Castro-Hermida *et al*. (2002)
Australia	Period prevalence	48.1	0-7 weeks	*C. parvum*	Becher *et al*. (2004)
Germany	Point prevalence	20-30	Up to 6 months	*C. parvum*	Joachim *et al*. (2003)
Canada	Period prevalence	100	0-4 months	*C. parvum*	O'Handley *et al*. (1999)
Canada	Period prevalence	5	<1 month	*C. parvum*	Ralston *et al.* (2003)
Canada	Period prevalence	0-40	Cows	*C. andersoni*	Ralston *et al*. (2003)
Canada	Point prevalence	15	0-6 months	Not reported	Olson *et al*. (1997b)
Canada	Point prevalence	9	>6 month	Not reported	Olson *et al*. (1997b)
Canada	Point prevalence	10.6	Cows	***C. muris****	McAllister *et al.* (2005)
Canada	Point prevalence	18.4	Cows	***C. parvum***	McAllister *et al*. (2005)

Table 1 continued

Country	Reported prevalence		Age reported	Species	Source
	Type	**Rate (%)**			
Canada	Point prevalence	13	2-70 days	**Not reported**	McAllister *et al*. (2005)
Czech Republic	Period prevalence	11.1-92.9	Calves	*C. andersoni*	Kvac and Vitovec (2003)
Czech Republic	Period prevalence	43.80	Cows	*C. andersoni*	Kvac and Vitovec (2003)
Poland	Point prevalence	24.56	4-27 days	Not reported	Pilarczyk and Balicka-Ramisz (2002)
Mexico	Point prevalence	25	1-30 days	***C. parvum***	Maldonado-Camargo *et al.* (1998)
England	Point prevalence	62.4	Adult cattle (1-15) years	***C. parvum***	Scott *et al*. (1995)
Japan	Period prevalence	93	1-30 days	*C. parvum*	Uga *et al*. (2000)
Denmark	Point prevalence	17	1-30 days	***C. parvum***	Henriksen and Krogh (1985)
Turkey	Point prevalence	65.8	Calves and adults	Not reported	Emre *et al*. (1998)
Brazil	Period prevalence	47	1-14 months	*C. muris**	De J. Pena *et al*. (1997)
Tanzania	Point prevalence	5.3	Calves and adults	Not reported	Mtambo *et al.* (1997)
France	Point prevalence	17.9-43.4	4-21days	Not reported	Lefay *et al*. (2000)
Scotland	Point prevalence	84	Adult cattle	***C. parvum***	Bukhari and Smith (1996)
Scotland	Point prevalence	16	Adult cattle	***C. muris****	Bukhari and Smith (1996)

* Has been renamed, *C. andersoni*

Bovine coccidiosis also occurs worldwide (Ernst and Benz, 1981; Radostits *et al.,* 1994; Kaufman, 1996; Urquhart *et al.*; 1996) but is of most importance where animals are housed or confined in small areas (Radostits *et al.*, 1994). In spite of the worldwide distribution, bovine coccidiosis is not a reportable disease in most countries (Ernst and Benz, 1981) and hence the

available data about its prevalence and the losses it causes are scant. Nevertheless, the reported prevalence ranges from 16% to 87.8% (Table 2).

Table 2. Prevalence of bovine coccidiosis in different countries of the world

Country	**Reported prevalence**		**Age affected**	**Source**
	Type	**Rate (%)**		
Poland	Point prevalence	27.1	Cows	Pilarczyk *et al*. (2000)
Poland	Point prevalence	49.6	Calves	Pilarczyk *et al*. (2000)
Netherlands	Period prevalence	46	Calves	Cornelissen *et al*. (1995)
Netherlands	Period prevalence	43	Yearlings	Cornelissen *et al*. (1995)
Netherlands	Period prevalence	16	Cows	Cornelissen *et al*. (1995)0
Kenya	Point prevalence	67.4	Calves and adults	Munyua and Ngotho (1990)
South Africa	Point prevalence	29-52	Calves and adults	Matjila and Penzhorn (2002)
Japan	Point prevalence	19.3	Not reported	Hasbullah *et al* (1990)
Japan	Point prevalence	59	2 weeks to adults	Oda and Nishida (1990)
Saudi Arabia	Point prevalence	34.1	Calves adults	Kasim and Al-Shawa (1985)
USA	Point prevalence	86.3	Calves	Ernst *et al*. (1987)
Turkey	Point prevalence	68	Calves and adults	Arslan and Tuzer (1998)
Mexico	Period prevalence	87.8	Calves	Rodriguez-Vivas *et al*. (1996)

2.5.2 Host range

Based on transmission experiments, *C. parvum* was shown to lack host specificity (Aiello and Mays, 1998; Radostits *et al.,* 1994; Urquhart *et al.,* 1996; Floyd, 2000: ISU, 2003; Fayer *et al.,*

2000) and, consequently about 152 species of mammals including humans are reported to be infected with the parasite (Fayer *et al.,* 2000). This cross species infectivity differentiates *C. parvum* from most other cryptosporidian protozoa, which are fairly host specific (Floyd, 2000). Among the domestic animals, cryptosporidiosis due to *C. parvum* is common in calves and other young ruminants, occurs in pigs, and is rarely seen in dogs, cats, and horses (Aiello and Mays, 1998; ISU, 2003). Genetically confirmed *C. andersoni* infection has been found only in cattle, Bactrian camels, and a sheep (Xiao *et al*., 1999).

All domestic animal species are susceptible to coccidial infections (Quigley, 2001; Radostits *et al.,* 1994) but unlike the cryptosporidia, coccidia are generally host-specific (Kirkpatrick and Selk, 2003; Radostits *et al,* 1994; Quigley, 2001; Adams and Perth, 2004) and infection does not pass readily from one animal species to another nor does cross-immunity between species of coccidia occur (Radostits *et al.,* 1994). However, each host may be infected with several species of coccidia at the same time (Quigley, 2001). All breeds of cattle are equally susceptible (Kennedy, 2001).

2.5.3 Transmission

Transmission of cryptosporidiosis, both within and between host species including humans, is by the fecal-oral spread of the environmentally resistant oocysts, which are fully sporulated and infective when excreted in feces. The most common route of infection is close contact with the diarrheic feces of infected individuals (Scott *et al.,* 1995).

The transmission of coccidiosis from animal to animal is also achieved by the fecal-oral route (Radostits *et al.,* 1994; Kirkpatrick and Selk, 2003; Adams and Perth, 2004). The source of infection is the feces of clinically affected or carrier animals (Ernst and Benz, 1981; Radostits *et al.,* 1994; Radostits, 2001), and the susceptible animal contracts the infection by ingesting feed or water, or by licking the hair coat contaminated with infected feces (Radostits *et al.,* 1994; Kirkpatrick and Selk, 2003).

2.5.4 Risk factors

Various studies have been conducted overseas to establish the factors associated with the risk of infection by either of the parasites in dairy herds. These factors are summarized as follows.

Age: It has been shown that there is a significant association between age and risk of infection with *Cryptosporidium*. According to the studies, cryptosporidiosis due to *C. parvum* is predominately a problem of neonate animals (Upton, 2003) with a maximum rate of excretion of oocysts between 4 and 21 days of age (Angus, 1990; Garber *et al*., 1994; Quilez *et al.,* 1996; Olson *et al.,* 1997b; de la Fuente *et al.,* 1999; O'Handley *et al.,* 1999). Although exceptions occur, older animals generally develop poor infections, even when unexposed previously to this parasite (Upton, 2003). Unlike *C. parvum*, *C. muris* (*C. andersoni*) was only found in weaned calves or adult cattle (Anderson, 1989; Bukhari and Smith, 1996; de J. Pena *et al.,* 1997; Wade *et al.,* 2000).

Although adult cattle are described as excretors of *C. parvum* oocysts, their importance as a source of infection for the young remains questionable because oocyst excretion by adult cattle was similar in herds with serious problems of cryptosporidial neonatal diarrhea and in those without (Scott *et al.,* 1995). Besides, studies have revealed that periparturient cows on dairies in which most young calves became infected and shed this parasite were not shedding detectable *Cryptosporidium* oocysts (Atwill *et al.,* 1998; Anderson, 1982).

Coccidiosis in cattle is primarily a disease of young cattle (Aiello and Mays, 1998; Quigley 2001; Kirkpatrick and Selk, 2003) although occasionally occurs in older animals that are in poor general condition (LAV, 1996). The disease is seen most frequently in calves that are 6 to 12 months of age (Kennedy, 2001).Unlike cryptosporidia, older animals usually serve as carriers of coccidia and continue to pass oocysts in the feces to the environment (Soulsby, 1982; Kennedy, 2001) which results in a build-up of infection in yards barns and on pasture, so that severe and fatal coccidiosis may result when a new batch of calves is placed on a pasture or in a yard, which hitherto has appeared perfectly safe (Soulsby, 1982).

Management and hygienic conditions*:* The risk of *Cryptosporidium* infection increases when animals are communally housed and over crowded (Angus, 1990; Atwill *et al.,* 1999; Mohammed *et al.,* 1999) and when hygiene and certain other management practices are deficient (Reynolds *et al.,* 1986; Atwill *et al.,* 1999; Mohammed *et al.,* 1999). *Cryptosporidium* infections were common in single or multiple suckler beef herds (Reynolds *et al.,* 1986) and dairy farms with multiple cow maternity facilities (Garber *et al.,* 1994). Overcrowding in unhygienic conditions is also a major risk factor that precipitates massive intake of *Eimeria* oocysts and leads to development of clinical coccidiosis (Radostits *et al.,* 1994; Ernst and Benz, 1986; Urquhart *et al.,* 1996).

Stress: Coccidiosis in feedlot cattle is associated with stress caused by shipping, sudden changes in ration and weather, and overcrowding (Ernst and Benz, 1986; Aiello and Mays, 1998; Dedrickson, 2002) whereas stress caused by weaning makes dairy calves very susceptible to coccidiosis (Rebhun, 1995, Aiello and Mays, 1988; Quigley, 2001). Coccidiosis can also occur in free-ranging conditions resulting from severe weather stress (Aiello and Mays, 1998) and crowding around a limited water source (Aiello and Mays, 1998; Troncy, 1989) as may occur during drought periods (Troncy, 1989), which concentrates the hosts and parasites within a restricted area (Aiello and Mays, 1998).

Herd size: An association between the size of the farm and the risk of infection with *Cryptosporidium* has been described by several authors (Garber *et al*., 1994; Quigley *et al*., 1994; Mohammed *et al*., 1999), where the higher the density of animals, the greater the number of calves which become infected and which in turn, contaminate their surroundings. Higher prevalence for coccidial infections has also been reported in large herds (Rodriguez-Vivas *et al*., 1996) related with the fact that calves of the large herds are in overcrowded conditions and are consequently more likely to ingest large numbers of sporulated oocysts over a short period of time.

Season: An increase in the prevalence of cryptosporidiosis during certain seasons has been reported by some authors, related with high rainfall or the number of births (Garber *et al*., 1994;

Atwill *et al*., 1999; Mohammed *et al*., 1999; Lefay *et al*., 2000). In a Canadian study of beef calves, higher prevalence was found in winter and spring, the period related to calving season and consequently the period with the greatest number of calves in the high-risk group (1-3 weeks old) (de Graaf *et al*., 1999). However, in American dairy farms, where calvings tend to be year round and environmental contamination level is less subjected to fluctuations, cryptosporidiosis was more prevalent in the summer (Garber *et al*., 1994).

Coccidiosis usually is sporadic during the wet seasons of the year, but may occur at any time in animals confined in feedlots. Severe losses have been reported in cattle confined in feedlots during periods of extremely cold weather (Ferguson, 1996; Aiello and Mays, 1998). As reported by Adams and Perth (2004), the disease can be a problem at any time of year so long as conditions of adequate moisture and temperature exist for survival and development of oocysts.

Resistance of the oocysts: *Cryptosporidium* oocysts are extremely resistant to environmental factors and to the action of chemical agents commonly used including chlorine (O'Donoghue, 1995; Martins and Guerrant, 1995; Gomez, 2001) and therefore, they are able to survive in the environment- bedding, walls, feeding troughs, drinking units, utensils, etc, and maintain their infective capacity for prolonged periods of time. This greatly facilitates diffusion of the disease (Gomez, 2001). The oocysts of coccidia are also fairly resistant to chemical disinfectants and detergents (LAV, 1996) but are sensitive to desiccation and high temperatures (Soulsby, 1982; Radostits *et al.*, 1994; LAV, 1996). They are destroyed immediately by boiling water or steam and within10 minutes at 50^{o}C. However they can remain in the environment (particularly moist, shady areas) for several years and maintain their infectivity (Adams and Perth, 2004) and can be stored for years at 4 to 5 ^{o}C but are destroyed by freezing (LAV, 1996).

2.6 Pathogenesis and clinical Signs

Cryptosporidiosis

Of the two species of *Cryptosporidium* that infect cattle, only *C. parvum* is more pathogenic and has been associated with neonatal diarrhea (de Graff *et al.*, 1999). In cattle, *C. andersoni* infects only the glands of abomasum and the infection is considered to be clinically mild affecting weigh gain and milk production (Anderson, 1987; Esteban and Anderson, 1995). As reported by Anderson (1987), *Cryptosporidium andersoni* usually causes no overt illness, but retards acid production. Protein digestion in the abomasum probably is retarded, and, in fact, milk production in cows that are chronically afflicted with *C. andersoni* is reduced about 13%. Growing calves may be adversely affected also.

Cryptosporidium parvum does not invade in to the cytoplasm and infections are mainly concentrated in the brush border (microvilli) cells of the distal small intestine (Aiello, 1998; Williamson, 2002). The pathological findings associated with *Cryptosporidium* are mild to severe intestinal villous atrophy, fusion, blunting, distortion and metaplasia of the surface epithelium. The villous blunting and fusion decreases absorptive surface area and microvillar disaccharidase secretion (Floyd, 2000). Diarrhea results from malabsorption and maldigestion, and increased secretary activity. The diarrhea is diffuse, watery and yellowish in color. The feces can contain undigested milk, blood, fibrin, and mucus. Moderate dehydration, mild-to-moderate depression, tenesmus, and low-grade fever are common signs. Chronically affected animals become emaciated. Most uncomplicated cases will recover in 6 to 10 days Relapses are fairly common, and can occur from auto-reexposure (Williamson, 2002). The disease typically causes high morbidity and low mortality (Kirkpatrick, 1985: Mann *et al*., 1986; Sobieh *et al.*, 1987; Scott *et al.*, 1995; Williamson, 2002).

Coccidiosis

Eimeria zuernii and *Eimeria bovis* are the two species of coccidia that are considered most pathogenic to cattle. They have similar life cycles, which involve two asexual and one sexual

phase; and second generation schizogony and gametogony are the stages in the lifecycle of these parasites that cause functional and structural lesions of the large intestine (Radostits and Stockdale, 1980; Radostits *et al.*, 1994).

Development of the second-generation schizonts and gamonts of both spp. within the epithelial cells causes mechanical disruption of the cytoplasm. This in turn prevents normal absorptive function of the caecal and colonic epithelia (Radostits and Stockdale, 1980). As the second generation schizonts or gamonts mature the cells containing them slough from the basement membrane and cause hemorrhage and destruction of the caecum and colon (Radostits and Stockdale, 1980; Radostits *et al.*, 1994). The oocysts are the result of fertilization of the gametocytes and are discharged at the time of rapture of the cells, which usually coincides with the onset of clinical signs of dysentery (Radostits *et al.*, 1994).

The severity of the disease depends on a number of factors such as the number of oocysts ingested (LAV, 1996; Kennedy, 2001), the species of coccidia present, and the age of the animal (Kennedy, 2001). Clinical signs of coccidiosis include profuse diarrhea (bloody at times), rectal straining (tenesmus), loss of appetite, slight fever, rough coat, debility and death in sever cases (LAV, 1996; Ferguson 1996; Radostits *et al.*, 1994). Severely affected calves may never regain productivity losses (DHM, 1998).

In coccidiosis, morbidity is higher than expected (Rebhun, 1995) because many infected animals (95%) remain sub clinical or show mild signs and clinical coccidiosis constitutes only 5% of all the cases (Quigley, 2001; Dedrickson, 2002). Mortality is low unless the problem is neglected, if severe oocyst loads exist, or if concurrent stress or disease simultaneously affects the coccidiosis patients (Rebhun, 1995). Exceptionally the mortality rate is higher in bovine winter coccidiosis accompanied by nervous sings. The pathogenesis of nervous coccidiosis is unknown, but more than 90% of the cases occur during the coldest month of the year and it may affect as many as one third (30%) of the cattle in some herd outbreak of coccidiosis, especially in beef cattle (Radostits and Stockdale, 1980; Bowman, 1999). In addition to acute diarrhea, affected animals display neurological syndrome such as muscular tremors, convulsions, opisthotonus and blindness, and a mortality rate of 50% (Radostits and Stockdale, 1980; Bowman, 1999).

2.7 Immunity

Cryptosporidiosis

A variety of immune mechanisms have been implicated in host resistance or susceptibility to infection, the modulation and eradication of active infections and the acquisition of protection against subsequent challenge (O'Donoghue, 1995). Active infections in immunocompetent hosts are generally self–limiting and result in partial or complete protection against subsequent challenge (O'Donoghue, 1995; Aiello and Mays, 1998; Abrahamsen, 1998). In contrast, severe chronic infections may develop in immunocompromised hosts with either congenital or acquired lymphocyte or gammaglobulin deficiencies thereby suggesting that both cell-mediated and humoral immune responses are involved in the resolution of infections and the development of protection. Other non-specific factors, such as host age and nutritional status, have been associated with increased susceptibility to clinical or chronic infections (O'Donoghue, 1995).

Coccidiosis

The immunity to bovine coccidiosis is not well understood, but is thought to be a combination of cellular and humoral factors (Urquhart *et al*., 1996; LAV, 1996). Cell-mediated immune responses appear to be more important than humoral responses, although antibodies are likely important in providing a certain degree of protection against recurrence of disease (LAV, 1996). Recovered calves are thought to be relatively immune to reinfection by the same species of *Eimeria* but at risk for infection by other species (Rebhun, 1995), as the immunity is species specific (Radostits *et al.,* 1994; LAV, 1996). For example, animals that are immune to *E. zuernii* are still susceptible to *E. bovis*- and strength of immunity is different for the various species. Breakdown in immunity associated with either severe challenge or stress-related relaxation of resistance do occur (LAV).

2.8 Diagnosis

Cryptosporidium infections can be diagnosed by: a) histological examination of autopsy or biopsy material (O'Donoghue, 1995); b) demonstration of oocysts in the feces by Sheather's sugar flotation technique (Hendrix, 1998; Bowman, 1999); c) microscopic staining methods, the stain of choice for many diagnostic laboratories being acid-fast staining (O'Donoghue, 1995; Fayer *et al*., 2000); d) immunological – based detection methods including polyclonal FAT, LAT, IF with MAbs, ELISA, and RPH (Fayer *et al.,* 2000); e) immunoserology using IF detection and ELISA, and solid-phase qualitative immunochromatographic assays (O'Donoghue, 1995; Fayer *et al.,* 2000); and f) molecular techniques using a variety of PCR tests (O'Donoghue, 1995; Fayer *et al.,* 2000).

Coccidiosis

Diagnosis of clinical coccidiosis can be made from a combination of herd history (Adams and Perth, 2004; Kennedy, 2001; Ferguson, 1996), clinical signs (Kennedy, 2001; Ferguson, 1996; Adams and Perth, 2004; Soulsby, 1982), gross lesions at necropsy (Ferguson, 1996) and identification of the oocysts in sugar flotation methods (Ernst and Benz, 1981; Bowman, 1999; Aiello and Mays, 1998), as well as fecal oocyst count (Adams and Perth, 2004).

2.9 Treatment, prevention and control

Cryptosporidiosis

Despite many antimicrobial agents have been tried and investigated for treating calves with cryptosporidiosis (Nydam, 2003), currently there are no specific and effective drugs labeled for elimination of the parasite (Aiello and Mays, 1998; Williamson, 2002). Of the drugs tried, halofuginone is one antimicrobial that has shown promise in Europe to treat and prevent cryptosporidiosis in dairy calves (Joachim *et al*., 2003; Nydam, 2003). In at least 3 trials with reasonable numbers of calves it has decreased oocyst shedding and improved fecal consistency

scores. Unfortunately, the drug is currently not available in the market (Nydam, 2003). Fluid therapy, nonsteroidal anti-inflammatory drugs, and good nursing care are important mainstays of therapy (Williamson, 2002).

Sanitation is the mainstay for control of this disease in calves. Proper manure handling and sanitation of calf housing and feeding equipment reduces the risk of disease in calves and decreases the number of organisms in the environment (Angus, 1990; Williamson, 2002).

With regard to prophylaxis with vaccination, recently a recombinant vaccine against specific proteins in the C. *parvum* sporozoite and merozoite stages has been developed that can be administered to pregnant cows. The vaccine has successfully induced immune colostrum in vaccinated cows, which reduced oocyst shedding by 99.8%, and fecal volume in calves that ingested it shortly after birth. The cows were vaccinated three times during late pregnancy. This vaccine is still in the development phase and is not commercially available (Perryman *et al*., 1999).

Coccidiosis

Treatment of bovine coccidiosis is very difficult because the clinical sings appear usually only when the life cycle of the parasite is advanced and marked destruction of mucosa may already have taken place (Soulsby, 1982; DHM, 1998; Bowman, 1999; Quigley, 2001; Dedrickson, 2002). Several medications are effective against coccidiosis if given early in the course of the disease, before symptoms appear. It is too late to halt the infection by the time clinical signs appear, but it is necessary to prevent later outbreaks. Supportive treatment may still be necessary to save calves, to prevent dehydration and to ward off possible secondary diseases that may result from weakened condition (Bowman, 1999; Dedrickson, 2002; Adams and Perth, 2004).

For the control of coccidiosis proper sanitation (Quigley, 2001; Adams and Perth, 2004) and good animal husbandry practices (LAV, 1996; Adams and Perth, 2004; Ferguson, 1996; Kennedy, 2001), and the prophylactic administrations of anticoccidial drugs (LAV, 1996; Bowman, 1999) are very important.

2.10 Public health importance of *Cryptosporidium*

Cryptosporidium infection is of public health importance because it can infect and cause disease in human. In humans, cryptosporidiosis is caused predominantly by *Cryptosporidium parvum* or *C. hominis* (the latter was previously known as the *C. parvum* human genotype) (Fayer *et al*., 2000). The infection is transmitted predominantly from person to person but direct infections through contact with infected animals or feces and water-borne infection from contamination of surface water and drinking water by domestic or wild animal feces can also be important (Radostits, 2001). *Cryptosporidium* can affect humans of all ages, although the disease is more common in children (Quigley, 2001)

Cryptosporidiosis in humans is characterized by profuse, watery diarrhea with cramping, abdominal pains, nausea, anorexia, flatulence and malaise. Some individuals may also experience vomiting, weight loss, fever or myalgia (ISU, 2003; Juranek, 1996). The disease is usually self-limiting in immunocompetent persons but may be chronic, debilitating and severe in those who are immunocompromised (e.g. AIDS patients) (Martins and Guerrant, 1995; Juranek, 1996; ISU, 2003). Outbreaks of cryptosporidiosis in patients who have AIDS are associated with a mortality rate that is higher than 50% (Eisen, 2004). Animal handlers on a calf farm can be at high risk of diarrhea due to cryptosporidiosis transmitted from infected calves (Radostits, 2001) and cross-transmission from calves to livestock handlers, including veterinarians and veterinary students has been documented and therefore, Veterinarians and other livestock handlers should use disposable gloves and even surgical masks in some situations to minimize ingestion or inhalation of oocysts (Corwin, 1992). Immunocompromised people should also be restricted from access to young animals and possibly from access to farms (Radostits, 2001).

2.11 Economic importance of cryptosporidiosis and coccidiosis

Although cryptosporidial infection leads to few deaths, serious economic losses can occur because of problems associated with the resulting diarrhea—dehydration, weight loss and slow growth (Sanford and Josephson, 1982)—and the costs involved in the treatment—oral

administration of electrolytic solutions and drugs—and the application of suitable hygiene practices (de Graaf *et al*., 1999).

Coccidiosis is also considered to be sufficiently important economically in calves to warrant control measures (Radostits *et al.,* 1994). Apart from its impact on the health of animals, the disease inflicts enormous economic losses to cattle producers as it damages the absorptive surface of the intestine and weaken the immune system, leading to poorer feed efficiency, slower weight gain, weigh loss, longer heifer development periods, increased susceptibility to other disease (DHM, 1998), and mortality that may reach 24% (Sinks *et al.,* 1992). In USA alone, coccidiosis costs cattle feeders more than $400 million annually in lost profits due to reduced feed efficiency, slower weight gain, and increased susceptibility to other diseases (Dedrickson, 2002) and in a 1990 study, the disease was ranked as the third most prevalent health problem of cattle, second only to pneumonia and IBR (DHM, 1998).

2.12 The status of cryptosporidiosis and coccidiosis in Ethiopia

Diarrhea is one of the most frequently reported causes of calf morbidity and mortality in Ethiopia followed by pneumonia (Lemma *et al*., 2001; Amoki, 2001; Wudu, 2004); however, there are only little reports as to the specific agents involved. There is only one report concerning the presence of *Cryptosporidium* in the country. This is a longitudinal study carried out by Wudu (2004) to determine the causes of calf morbidity and mortality. He reported *Cryptosporidium* with a prevalence of 6.7% in diarrheic calves 20 to 90 days of age in small and large scale dairy farms located in Debre-Zeit.

With regard to coccidia there are two reports from different parts of the country. These are a 20% prevalence report in calves over 2 months of ages in small-scale dairy farms at Debre Zeit (Kebadu, 1998) and a five-year retrospective laboratory report of 24.9% from Bahr Dar Regional Veterinary Laboratory (Kassa *et al*., 1985). In addition to the above reports an outbreak of coccidiosis caused by *E. zuernii* was also reported in 9 calves and 2 cows with severe diarrhea in Abay Tana settlement dairy farm in Bahr Dar (Kassa *et al*., 1985).

3. MATERIALS AND METHODS

3.1 Description of the study area and study population

3.1.1 Study area

This study was carried out on selected urban dairy farms located in Addis Ababa city and Debre Zeit town. The criteria for selection of these sites were that they represent two goegraphical zones: highland (Addis Ababa) and midland (Debre Zeit), and also their proximity to laboratory. Addis Ababa is the capital city of the Federal Democratic Republic of Ethiopia. Geographically, the city is situated in a high land area with an altitude of 2500 meters above sea level. It has an average annual rainfall of 1800 mm, an average temperature of 21^0c and a relative humidity varying from 70% to 80% during the rainy season and from 40% to 50% during the dry season. Addis Ababa has an estimated human population of 3 million (CSA, 2001).

Debre Zeit is a town located about 45 km South East of Addis Ababa at an altitude of 1850 meter above sea level. It has a total human population of 95,000. The area experiences a bimodal rainfall pattern with a short rainy season from March to May and a long rainy season from June to October. The area has an average annual rainfall of 800mm and average maximum and minimum temperature of 27.7^{o}C and 12.3^{o}C, respectively (CSA, 2001). The town is the center of Ada'a Liben woreda administration in East Showa zone of Oromiya regional state and according to the information obtained from Ada'a Liben woreda agricultural and rural development office, the woreda has a total land area of 161.056 km^2

3.1.2 Study population

The target population constitutes all dairy farms found in Addis Ababa and Debre Zeit. According to the data obtained from the Agricultural offices of the respective areas, there are about 152 private owned commercial scale dairy farms that posses 10 and above cows per farm, and 2 farms that belong to governmental and non-governmental research centers. However, data

about the herd composition of the farms on age basis were lacking. The sampling frame for the study included these 154 farms. The study population constitutes all calves less than one year of age in the dairy farms in both areas.

3.2 Study design

The study design used was a cross-sectional study but sampling was made at different seasons in order to see seasonal variation in the prevalence of the parasites.

3.3 Sampling methods and determination of sample size

The sampling method employed was a one-stage cluster sampling where a sample of clusters (dairy farms) were selected first and then calves less than 1 year of age in the selected farms were all sampled. The total number of dairy farms included in the sampling frame was 154 and the average number of calves less than 1 year of age per cluster was predicted to be 15.

The numbers of clusters (dairy farms) required for the study were calculated based on the formula given by Thrusfield (1995) for one-stage cluster sampling method as follows:

$$g = \frac{1.96^2 \{nVc + Pexp(1-Pexp)\}}{nd^2}$$

Where**:**

g = number of clusters to be sampled,

n = predicted average number of calves per cluster,

Vc = between-cluster variance component (the variation expected between clusters if all animals in the clusters were sampled),

Pexp= expected prevalence (an overall mean cluster prevalence anticipated), and

d = desired absolute precision

At the beginning of the study the average number of calves per cluster (farm) was predicted to be 15. Since there were no well-documented similar studies done in Ethiopian dairy farms, the expected overall mean prevalence (Pexp) was taken as 32.5% by taking the average of the findings of similar studies overseas. As data derived from previous cluster samples were not available, the between-cluster variance component (Vc) was obtained by guessing (Thrusfield, 1995). Thus, with anticipated overall mean cluster prevalence of 0.325 (32.50%), and the average between this and the individual cluster prevalence was guessed to be 0.105 (10.5%), then Vc will be $0.105^2 = 0.011$. Therefore, with 95% confidence interval and 5% desired absolute precision, the number of clusters (farms) calculated was 39, and with average number of 15 calves per cluster, a total of 585 calves were needed for sampling. However, the number of farms was increased to 40 since the required number of calves was not obtained in 39 farms.

Before sampling the farms were stratified into three categories based on the total number of animals they own as: small (<50), medium (50 to 150) and large (>150). Then, the required numbers of farms were selected proportionally from the three strata by random sampling method, and all the calves in these selected farms were included in the study (Table 3). The farms were randomly assigned for sampling within the three seasons of study period. Each farm was visited once during the study period from September 2004 to March 2005 to collect fecal samples and conduct questionnaire survey.

Table 3. Number of farms and calves sampled from the two study sites

Herd size	**No of Farms selected**			**No of calves sampled**								
	Debre Zeit	**Addis Ababa**	**Total**	**Debre Zeit**			**Addis Ababa**			**Total**		
				Male	Female	Total	Male	Female	Total	Male	Female	Total
Small	2	28	30	8	20	28	35	150	185	43	170	213
Medium	4	3	7	33	55	88	4	44	48	37	99	136
Large	1	2	3	64	70	134	22	75	97	86	145	231
Total	7	33	40	105	145	250	61	269	330	166	414	580

3.4 Sample collection

A fresh fecal sample of approximately 30 gm was collected from each calf by retrieval per rectum, using disposable plastic gloves new for each calf. The sample was placed in a separate disposable plastic container and transported in a cool box to the laboratory on the same day of collection, and preserved at refrigeration temperature until processing within 48 hours of arrival. At the time of sampling, the name of the farm, date of sampling, consistency of the feces (soft, pasty, watery or normal), and the age, sex, breed, and tag no of the calves were recorded for each calf on a recording format.

3.5 Laboratory investigation

3.5.1 Fecal examination with centrifugal flotation technique

This qualitative examination was conducted for the detection of the oocysts of *Cryptosporidium* and *Eimeria spp*. using concentrated sucrose solution (Sheather's sugar solution) with specific gravity of 1.27 as described by Hendrix (1998) as follows:

Approximately 2-3 g of feces was mixed with enough water in a plastic cup using tongue depressor to make a semisolid suspension. The mixture (feces and water) was strained through a tea strainer over a second plastic cup pressing out the liquid with tongue depressor. The contents of the second plastic cup was transferred in to a 15-ml centrifuge tube which then placed in to the centrifuge and spun for 3 minutes at 1500 revolutions per minute (rpm), processing 4 samples at a time. Then the supernatant, which contains fats and dissolved pigments, was decanted and the sediment was resuspended using a stirring action with a wooden applicator stick after adding the flotation solution to 1/2 inch from the top of the tubes. The test tubes were inverted six times after inserting a rubber stopper to mix the solution thoroughly with the sediment. The tubes were then filled with flotation solution until a reverse meniscus was present; cover slips were added and centrifuged in variable-angled (not a fixed-angled) centrifuge for 5 minutes. After centrifugation, the cover slip was lifted straight up and placed with its adherent film of sugar solution on a glass slide. The slides were examined under the compound microscope using the

10x objective for the identification of *Eimeria* oocysts, and 40x objectives for *Cryptosporidium* oocysts.

Cryptosporidium oocysts were detected in the sample with the correct morphology, i.e. optical properties, internal structure, size and shape. Criteria used to identify the two common species in cattle were: *C. parvum* oocysts measure 4-6 μm, are spherical with a residuum and sporozoites, and refract pink in Sheather's sugar; and *C. andersoni* oocysts measure 7-9 μm in length, are oval with a residuum and sporozoites, and refract pink in Sheather's sugar on bright-field microscopy (Wade *et al*., 2000). The identification of *Eimeria* species was based upon the characteristics of the sporulated oocysts of cattle given in Annex 1, which includes the size, shape, color and texture of oocyst wall, presence or absence of micropyle, polar cap, and, time of sporulation as described by Levine (1985) and Soulsby (1982). Size measurement of both parasites was done with calibrated ocular micrometer.

3.5.2 Fecal culture of coccidian oocysts for sporulation

For the identification of the different species of *Eimeria* and also to differentiate oocysts of *Eimeria* from those of *Isospora*, whose unsporulated oocysts have similar shape and size with *Eimeria*, those positive samples to coccidia oocysts in fecal flotation with more than 500 OPG were allowed to sporulated in 2.5% (w/v) potassium dichromate solution following the procedure described by Hendrix, C.M. (1998). 10 to 20 g of coccidia-positive sample was placed in a beaker and covered with about 60 ml of 2.5% potassium dichromate solution. The solution was mixed thoroughly with a tongue depressor and poured in a thin layer in a petridish and incubated at room temperature for a week. The plates were opened daily and the contents swirled gently to allow air to reach the developing oocysts. A small sample from the plates was taken daily to see whether sporulation has taken place and the time of sporulation was recorded. After incubation, the plate's content was centrifuged and the fecal sediment was processed by the centrifugal flotation procedure to recover the oocysts. A fully sporulated oocyst of the genus *Eimeria* contains four sporocysts, whereas a fully sporulated oocyst of the genus *Isospora* has two sporocysts.

3.5.3 Modified Kinyoun acid-fast staining

Since the oocysts of *Cryptosporidium* are so minute they can be confused in fecal flotation with some organisms like yeasts, which are about the same size and shape as *Cryptosporidium (*Aiello and Mays, 1998). Therefore, a modified Kinyoun acid-fast staining was done to increase the optical contrast and to stain confusing yeasts differentially according to the procedures described by Baron *et al*. (1994) as follows: An aliquot of 10% formalinized feces was spun for 10 minutes at 2500rpm and the upper layer of the sediment was removed with pipette, and a thin layer was placed on to a microscope slide. The smear was heat fixed at 70^{o}c for 10 min and stained with Kinyoun carbolfuchsin for 5 minutes. It was then washed in distilled water and flooded with a docolorizer for approximately 1 min; more docolorizer was added for very thick slides or those that continued to bleed red dye; and then washed thoroughly with distilled water as above, shaking off excess. Finally the slides were flooded with Kinyoun counterstain for approximately 1 min; washed with distilled water, drained by standing slides upright, and examined under compound microscope using 100x oil immersion lens. *Cryptosporidium* oocysts were stained bright red in the smear whereas other organisms and the background stained with the blue counter stain.

3.5.4 Quantitative Fecal Examination

This was performed to determine the number of oocysts of *Eimeria* per gram of feces (OPG). The method used for this purpose was the well-known McMaster technique as described by Kaufman (1996) as follows: 3 g of feces was weighed in a plastic cup and mixed with 42 ml of water. The mixture was homogenized and poured through a 250 um aperture sieve. The filtrate was agitated and a 15 ml (1 gm) suspension was drawn and filled in to a 15 ml centrifuge tube. The sample was centrifuged at 2000 rpm for 2 minutes, processing 4 samples at a time. The supernatant was poured off and concentrated sugar solution added to the sediment which then agitated adding the sugar solution to the previous level. Then the tube was inverted 6 times and both chambers of McMaster slide were filled quickly with fluid removed from the tube with pipette. The slide was scanned under 10x magnification and the oocysts counted in both chambers were multiplied by 50 to arrive at the number of oocysts per gram of feces (OPG).

3.6 Questionnaire survey

A well-structured questionnaire was designed and administered to collect data on demographic, management, hygiene and other factors hypothesized to be associated with the risk of infection with either *Cryptosporidium* or *Eimeria* species in dairy herds (Annex II). The demographic data included herd-size, age distribution, and breed of animals. Management factors included in the questionnaire were maternity facilities, calf feeding and watering system, housing condition and others. Data were collected by personal interview of the farm owner/manager.

3.7 Data analysis

The data collected from the two study sites were entered into Excel spreadsheet (Windows) and analyzed with SPSS for Windows (Version 11.5) and Stata for Windows (Version 7) statistical soft wares. The point prevalence for both parasites was calculated for all data as the number of infected individuals divided by the number of individuals sampled × 100. Categorical data were analyzed first with the Chi-square ($\chi 2$) test for independence as a screening process. This test was followed by stepwise multivariate logistic regression, to account for confounding variables and interactions. For analysis of continuous data, t-test for independent samples was used to compare means of two groups whereas ANOVA was used to compare means of three or more groups. A P value < 0.05 was required for significance. Odds ratios (OR) were determined from the coefficients in the logistic regression.

4. RESULT

4.1 Overall prevalence of *Cryptosporidium* and *Eimeria spp*.

Out of the 580 fecal samples examined, 102 (17.60%) and 395(68.10%) calves were positive for *Cryptosporidium* and *Eimeria* oocysts, respectively. Of these, 78 (13.45 %) calves were positive for both parasites (Table 4). Of the 40 dairy farms surveyed, 26 (65%) had at least one calf shedding *Cryptosporidium* oocysts, whereas all the farms (100%) had one or more calves shedding *Eimeria* oocysts.

Table 4. Point prevalence of *Cryptosporidium* and *Eimeria* spp. infection displayed on the basis of geographic zones

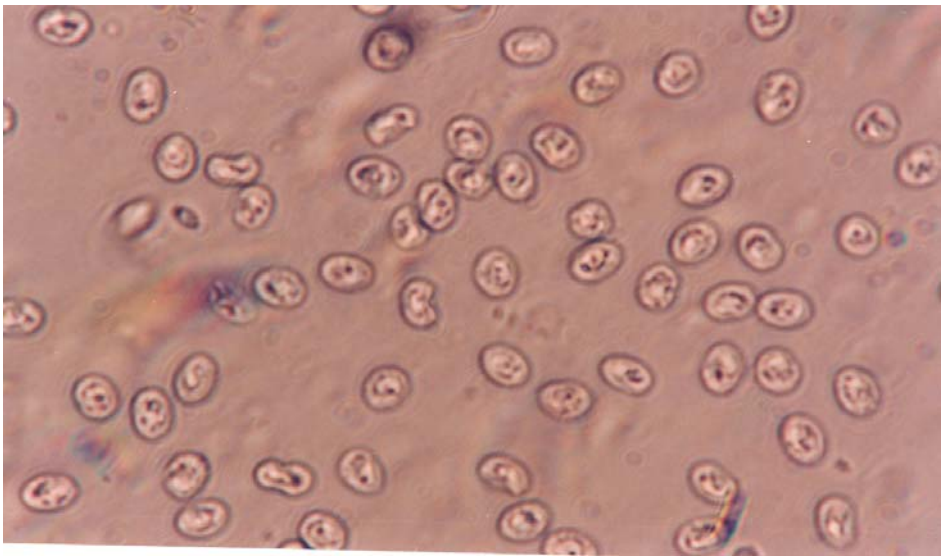
Geographical zone	No calves sampled	*Cryptosporidium spp*			*Eimeria spp*			*Mixed infection*		
		%Pos	95% CI		%Pos	95% CI		%Pos	95% CI	
Midland (Debre Zeit)	250	14	9.7	18.3	57.2	51.1	63.3	10.4	6.6	14.2
Highland (Addis Ababa)	330	20.3	16	25	76.4	72	81	15.8	11.9	19.7
Overall	580	17.6	16.3	21	68.1	64.3	72	13.5	10.7	16.23

4.2 Results of species identification

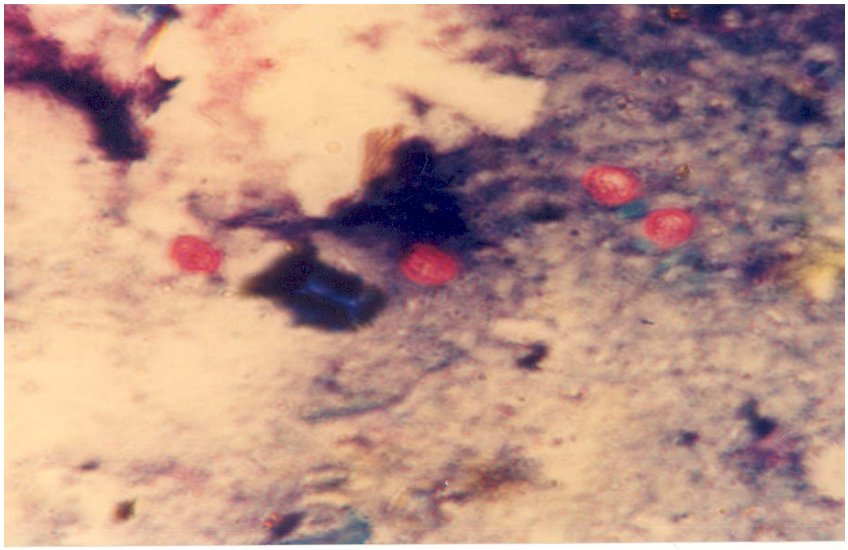
The species of *Cryptosporidium* identified in this study was presumed to be *C. andersoni* based on the morphology of the oocysts and the epidemiology of the parasite. The oocysts were ellipsoid in shape with sporozoites, pink-tinged in Sheather's sugar solution and measured 7 x 5.4 (6-8 x 5.2 - 5.6) μm in size (Figure 1).

Figure 1. *Cryptosporidium* oocysts identified from calves during the study period

a) Sheather's sugar solution demonstrating oocysts of *C. andersoni* (x1000)



b) Acid-fast staining of fecal smear(x1000)



With regard to identification of *Eimeria* species, a total of 11 species were identified of which *E. bovis* (38.45%) was the most prevalent species followed by *E. zuernii* (18.1%) and *E. auburnensis* (17.93%). *E. brasilensis* (1.21%) was the least prevalent species found (Table 5). Of the 395 calves positive for *Eimeria*, 45.60% were infected with single species, while the rest 54.43% were found to be infected with 2-7 species (Table 6).

Table 5. *Eimeria* species identified and their respective prevalence in descending order

Type of *Eimeria spp*.	**Total examined**	**No Pos.**	**(%)Pos**
E. bovis	580	223	38.50
E. zuernii	580	105	18.10
E. auburnensis	580	104	17.93
E. canadensis	580	98	16.90
E. ellipsoidalis	580	65	11.21
E. subspherica	580	39	6.72
E. cylindrica	580	37	6.40
E. alabamensis	580	27	4.70
E. wyomingensis	580	25	4.31
E. bukidnonensis	580	18	3.10
E. brasilensis	580	7	1.21

Table 6. Number of *Eimeria* species found per sample in infected calves

No of *Eimeria spp*. found	**No of calves**	**Relative occurrence (%)**
One	180	45.60
Two	127	32.20
Three	53	13.42
Four	24	6.10
Five	8	2.03
Six	2	0.51
Seven	1	0.25
Total	395	100

4.3 Results of the analysis of different factors with the risk of infection

There was a significant association between the agro-climatic zone and the risk of infection with either *Cryptosporidium* ($\chi 2 = 3.90$, $P = 0.048$) or *Eimeria spp*. ($\chi 2 = 24.10$, $P<0.001$) in chi-square test for independent samples. Age of the calves was strongly associated ($\chi 2 = 22.85$, $P < 0.001$) with the risk of infection with *Eimeria spp* but not associated ($\chi 2 = 1.90$, $P > 0.05$) with *Cryptosporidium*. The oocysts of both *Cryptosporidium* and *Eimeria spp*. were recovered from calves of a wide range of age i.e. in calves ranging from 0 to 345 days of age. The first age at which the oocysts of *Cryptosporidium* were detected was 21days and for that of *Eimeria spp*. was 15 days. A significant association also was observed between the feeding system of calves and the risk of infection with either *Cryptosporidium* ($\chi 2 = 8.7$, $P = 0.003$) or *Eimeria spp* ($\chi 2 = 7.11$, $P = 0.008$) (Tables 7 and 8).

As indicated in tables 7 and 8, season of the year when calves were sampled was not significantly associated with the risk of infection with either *Cryptosporidium* ($P > 0.05$) or *Eimeria spp* ($P > 0.05$). Herd size was significantly associated with the risk of infection with *Cryptosporidium* ($P = 0.018$) but not with *Eimeria spp*. ($P>0.05$). Analysis of the association between pre weaning calf-housing condition and risk of infection with the parasites showed a significant association with *Cryptosporidium spp.* ($P = 0.001$) but not with *Eimeria spp*. ($P>0.05$). The frequency of cleaning calf rearing houses was strongly associated ($P < 0.001$) with the risk of infection with *Cryptosporidium* but not significantly associated ($P > 0.05$) with the risk of infection with *Eimeria spp*. The method of cleaning also was significantly associated ($P = 0.047$) with the likelihood of infection with *Cryptosporidium* but not with *Eimeria* infection ($P > 0.05$).

Of the 580 calves sampled 121 were diarrheic, 124 had soft feces, and 335 were non-diarrheic and in analysis for association between fecal consistency and infection, neither *Cryptosporidium* ($P>0.05$) nor *Eimeria spp* ($P>0.05$) infection was found to be significantly associated with diarrhea (Table 9).

Table 7. Results of chi-square (χ^2) analysis of different risk factors for *Cryptosporidium* infection in dairy calves

Risk factor	No. exam	No. Pos (%)	OR	95% CI of OR		P	χ2	df
Geographical zone								
Midland (Debre Zeit)	250	14	1					
Highland (Addis Ababa)	330	20.3	1.6	1	2.45	0.048	3.90	1
Age in months								
0-6	381	16	1					
>6-12	199	20.6	1.4	0.88	2.11	0.168	1.90	1
Feeding system								
In feed troughs	436	15	1					
On the ground	144	26	2	1.3	3.12	0.003	8.70	1
Cleaning method								
Washing	380	15.5	1.0					
Sweeping only	193	22.3	1.6	1	2.42	0.047	3.99	1
Frequency of cleaning								
Daily	440	13.6	1					
Weekly	104	28	2.5	1.5	4.1	0.001		
Monthly	29	45	5.2	2.4	11.2	0.000	26.92	2
Season								
End of rainy	251	15.14	1					
Early dry	193	20.21	1.42	0.87	2.32	0.163		
Late dry	136	18.4	1.26	0.73	2.2	0.410	2.01	2
Herd size								
Large	231	14.3	1.0					
Medium	136	14.7	1.03	0.57	1.90	0.912		
Small	213	23.0	1.80	1.1	2.92	0.019	6.83	2
Preweaning housing								
Individual pen	353	14.45	1.0					
Group pen	165	20	1.5	0.91	2.40	0.112		
Tethered	56	32.14	2.8	1.50	5.29	0.001	11.15	2

Table 8. Results of Chi-square analysis of different risk factors for *Eimeria spp.* infection in dairy calves

Risk factor	No	No. Pos (%)	OR	95% CI of OR		P	$\chi 2$	df
Geographical zone								
Midland (Debre Zeit)	250	57.2	1					
Highland (Addis Ababa)	330	76.4	2.4	1.7	3.5	0.000	24.1	1
Age in months								
0-6	381	61.4	1					
>6-12	199	81	2.7	1.8	4.0	0.000	22.85	1
Feeding system								
In feed troughs	436	65.1	1					
On the ground	144	77.1	1.8	1.2	2.8	0.008	7.11	1
Cleaning method								
Washing	380	67.4	1					
Sweeping only	193	69.4	1.2	0.76	1.6	0.617	0.25	1
Frequency of cleaning								
Daily	440	67.7	1					
Weekly	104	66.4	0.94	0.6	1.5	0.787		
Monthly	29	79.3	1.83	0.7	4.6	0.200	1.85	2
Season								
End of rainy	251	63.75	1					
Early dry	193	71.5	1.43	0.95	2.14	0.085		
End of dry	136	71.32	1.41	0.90	2.22	0.133	3.87	2
Herd size								
Large	231	70.56	1.0					
Medium	136	56.62	0.54	0.35	0.85	0.007		
Small	213	72.77	1.11	0.74	1.7	0.606	0.188	2
Preweaning housing								
Individual pen	353	66.6	1.0					
Group pen	165	69.1	1.12	0.75	1.67	0.569		
Tethered	56	75	1.51	0.79	2.87	0.212	1.682	2

Table 9. Logistic regression analysis of the association between fecal consistency and the risk of infection with either *Cryptosporidium* or *Eimeria spp*

Description	No	%Pos	Odds Ratio	Std. Err.	Z	P>|z|	[95% CI for OR]	
Cryptosporidium spp.								
Diarrhea	121	7.44	1					
Soft	124	19.40	3.0	1.24	2.64	0.008	1.33	6.73
Normal	335	20.60	3.23	1.20	3.15	0.002	1.60	6.70
Eimeria spp.								
Diarrhea	121	53.00	1					
Soft	124	72.60	2.40	0.64	3.16	0.002	1.40	4.01
Normal	335	72.00	2.30	0.50	3.77	0.000	1.50	3.51

Figure 2. Point prevalence of *Cryptosporidium* and *Eimeria spp*. in different age groups

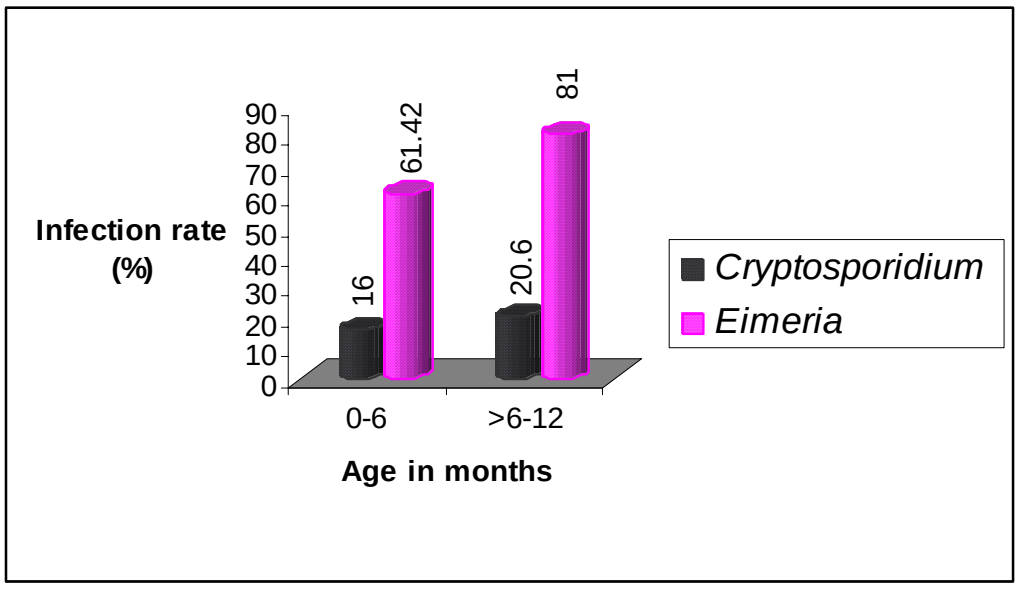


Figure 3. Point prevalence of *Cryptosporidium* and *Eimeria* infection displayed on the basis of frequency of cleaning calf houses

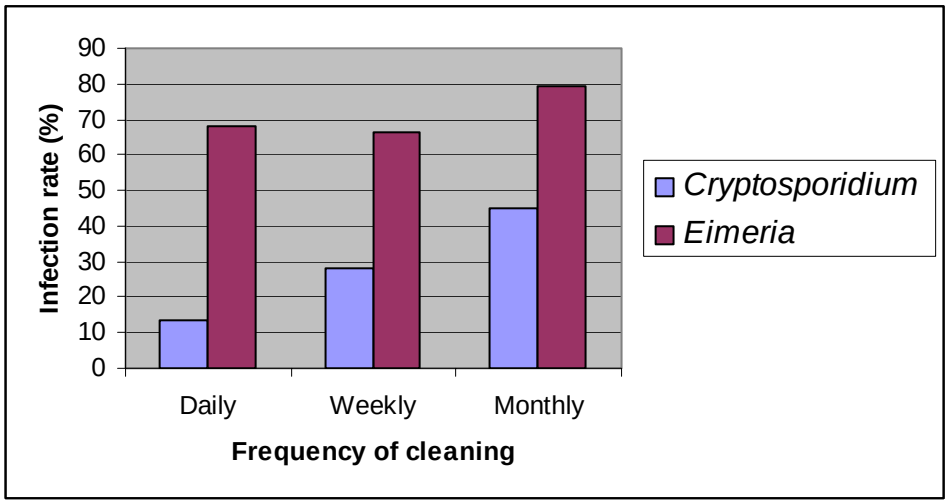


Table 10. Multivariate logistic regression analysis of risk factors associated with either *Cryptosporidium* or *Eimeria spp*. infection

Risk factor	Coefficient	Std. Err.	Z	P>|z|	[95% CI for Coefficient]	
Cryptosporidium						
Geographical zone	0.02	0.04	0.52	0.602	-0.05	0.09
Age in months	-0.01	0.04	-0.22	0.825	-0.08	0.06
Feeding system	0.04	0.05	0.86	0.388	-0.05	0.13
Season	0.03	0.02	1.42	0.155	-0.01	0.07
Herd size	-0.01	0.03	-0.40	0.692	-0.07	0.04
Preweaning housing	0.06	0.04	1.53	0.126	-0.02	0.13
Frequency of cleaning	0.695	0.24	2.88	0.004*	0.22	1.17
Cleaning method	-0.605	0.05	-2.10	0.085	-0.21	-0.01
Eimeria						
Geographical zone	0.26	0.04	3.88	0.032*	0.023	0.51
Age in months	0.24	0.04	3.98	0.031*	0.022	0.45
Feeding system	0.04	0.05	0.77	0.443	-0.06	0.15
Season	0.02	0.03	0.80	0.423	-0.03	0.07
Herd size	-0.03	0.03	-1.00	0.319	-0.10	0.03
Preweaning housing	0.001	0.04	0.03	0.979	-0.08	0.09
Frequency of cleaning	-0.03	0.05	-0.62	0.537	-0.14	0.07
Cleaning method	0.03	0.06	0.54	0.587	-0.09	0.15

* The association is significant at 0.05 level.

When all the risk factors which had significant associations with the risk of infection with either of the parasites in the first screening tests (univariate analysis) were further analyzed with multiple (multivariate) logistic regression, only the frequency of cleaning calf rearing houses (P = 0.004) was found to be significantly associated with *Cryptosporidium* infection, whereas, geographical zone (P = 0.032) and age of the calves (P = 0.031) were significantly associated with *Eimeria spp*. infection (Table 10). In final analysis, the interaction between geographic zone and age of the calves was not significant (Table 11). Therefore, the best fitting model that is biologically reasonable to describe the relationship between the risk factors and the likelihood of infection with either of the parasites is presented as follows:

For *Cryptosporidium* infection: logit (*p*) = -8.95+0.625 frequency of cleaning calf houses.

For *Eimeria spp* infection: logit (*p*) = -7.45+0.25geographical zone+0.23age of the calves.

Table 11. The best fitting model analyzed with logistic regression

Factor	**Coefficient**	**Std. Err**	**Z**	**Pr >|z|**	**[95% CI of Coef.]**	
Cryptosporidium						
Frequency of cleaning	0.625	0.137	4.57	0.000	0.357	0.893
Constant	-8.95	0.233	-38.45	0.000	-9.407	-8.49
Eimeria						
Geographic zone	0.25	0.11	2.36	0.018	0.042	0.46
Age	0.23	0.10	2.25	0.025	0.030	0.44
Geo. Zone*Age	-0.26	0.22	-1.18	0.237	-0.68	0.17
Constant	-7.45	0.21	-34.94	0.000	-7.89	-7.05

4.4 Results of quantitative fecal examination

The McMaster technique applied to determine the number of *Eimeria spp*. oocysts per gram of feces (OPG) revealed minimum and maximum OPG values of 0 and 267000, respectively. The maximum OPG belonged to a 24 days old diarrheic female calf. The mean, median and mode

OPG values were 5109, 800, and 200 respectively. The summarized result of OPG count was presented in Table 12.

There was a highly significant association ($t = 2.72$, $P = 0.007$) between age of the calves and OPG of *Eimeria spp.* but there was no association ($t = 0.838$, $P > 0.05$) between agro-climatic zone and OPG when tested with t-test for independent samples (Table 12). A One-way ANOVA test for association between fecal consistency and OPG showed a highly significant association ($F = 8.075$, $P<0.001$), with a highly significant mean difference between diarrheic calves and calves with normal feces ($P< 0.001$). There was also a highly significant mean difference between diarrheic calves and calves with soft feces ($P = 0.001$) (Table 14). On the other hand, a significant association was not seen between OPG and season of sampling ($F=1.531$, $P=0.218$).

Table 12. Grouped frequency distribution of *Eimeria spp*. oocysts counted per gram of feces (OPG)

OPG value	No. Calves	Percentage
0-100	28	11.20
101-1000	117	46.80
1001-2000	44	17.6
2001-3000	23	9.2
3001-4000	6	2.4
4001-5000	6	2.4
5001-10000	9	3.6
10001-20000	5	2
20001-30000	3	1.2
40000-50000	3	1.2
60000-70000	2	0.8
80000-90000	1	0.4
100000-200000	2	0.8
201000-300000	1	0.4

Table 13. T-test analysis of the association between age and geographical zone with OPG of *Eimeria spp*.

Risk factor	Mean	Std. Dev	Std. Error Mean	Mean Differ	95% CI of the Differ.		t	df	P
Age of calves in months									
0-6	8463	28574.5	2468.5						
>6-12	1235	1594.9	148.1	7228	1994	12462	2.72	248	0.007
Geographical zone									
Midland (Debre Zeit)	6914	17400	2080						
Highland (Addis Ababa)	4407	22538	1680	2506	-3385	8397	0.838	248	0.403

Figure 4. Distribution of *Eimeria* OPG in different age groups

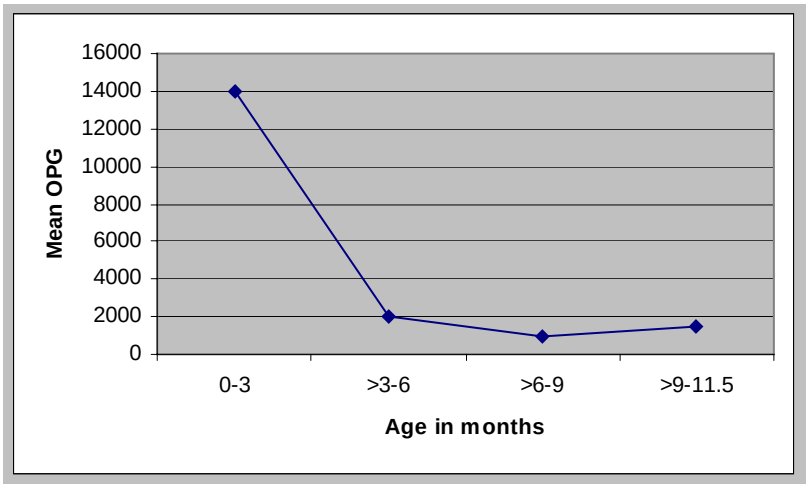


Table 14. Test for association between fecal consistency and mean OPG using one-way ANOVA

Fecal consistency	No calves	Mean OPG	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Mean Difference	P-value
Normal	166	2943	10686	829	1305.5	4581		
Soft	42	2018	4295	663	679	3356	925	0.795
Diarrhea	42	16761	45707	7053	2518	31004	13818	0.000
Overall	250	5109	21221	1342	2466	7752.50	F = 8.075	0.000

5. DISCUSSION

5.1 *Cryptosporidium* species

The species identified in this study was presumed to be *C. andersoni* based on morphometrics and observation of the epidemiology of the parasite. All morphological characters fit the description of *C. andersoni* (*C. muris*) given by several authors overseas (Wade *et al*., 2000; de J. Pena *et al*., 1997; Lindsay *et al*., 2000). The size measurements reported by these authors and others is 7.4 x 5.5 (6.0 to 8.1 x 5.0 to 6.5) µm. Therefore, the result of the present size measurement falls within this range. *Cryptosporidium parvum*, which is more pathogenic and common cause of calf diarrhea, was not found in this study. It is spherical in shape, with a residuum and sporozoites, and measures 5 x 4.5µm in size (Upton and Current, 1985; Fayer and Ungar, 1986). It is predominately a problem of neonate animals (Upton, 2003) with a maximum rate of excretion of oocysts between 4 and 21 days of age (Angus, 1990; Quilez *et al.,* 1996; Olson *et al.,* 1997; de la Fuente *et al.,* 1999) and infection is most often associated with diarrhea (de Graaf *et al*., 1999).

The absence of *C. parvum* has also been reported by one previous study (Abraham *et al*., 1992), who did the first formal study on agents associated with neonatal diarrhea in Ethiopian dairy farms and reported the presence of coronavirus, rotavirus, and ETEC from diarrheic calves up to 8 weeks of life. The only available report with regard to the presence of *Cryptosporidium* is that of Wudu (2004) who reported 6.7% point prevalence in dairy calves of Debre Zeit, comparatively lower than the present finding. However, whether the identified species is *C. parvum* or *C. andersoni* is not known. Besides this, the study subjects involved were all diarrheic calves that were less than 6 months of age.

When compared to the available few reports overseas that are concerned with *C. andersoni* (*C. muris*), the present finding is closely is similar with the prevalence reported in adult cattle (16%) in Scotland (Bukhari and Smith, 1996). In USA, relatively a lower prevalence (1.03-1.1%) was reported in calves (Anderson, 1989; Wade *et al*., 2000) but a wide range of prevalence (0.5-31%)

was reported in adults and unreported age group (Anderson, 1987; Anderson, 1989; Anderson, 1991). In calves period prevalences of 47% and 11.1-92.9% were reported in Brazil (de J. Pena *et al*., 1997) and Czech Republic (Kvac and Vitovec, 2003), respectively while 0-40% was reported in cows in Canada (Ralston *et al*., 2003) following a longitudinal study.

The lower prevalence of *Cryptosporidium* infection observed in this study as well as in many other similar studies is partly attributed to the type of design employed in studying the problem. The cross-sectional study design in general provides a momentary picture of the infection status of an individual herd (Thrusfield, 1995) and may underestimate the prevalence because oocyst excretion in *Cryptosporidium* infection can be intermittent, and the oocysts may be shed for only a few days (Casemore *et al*., 1997; McCluskey *et al*., 1995), consequently there was a possibility of missing calves that were infected at sampling.

Age of the calves was not significantly associated ($P > 0.05$) with the likelihood of infection with *C. andersoni*, although a higher infection rate was observed in calves 6-12 months of age (20.6%) than calves 0-6 months of age (16%). The infection rate was the least among calves 0-3 months of age when compared to other age group of calves; however, the present finding indicates that *C. andersoni* is important in all age groups of calves. In contrast to this study significantly higher infection rate has been reported in calves over 6 months of age by some authors (Wade *et al*., 2000). In the present study, *C. andersoni* was detected in calves aged as early as 3 weeks of age and this was found to be much lower than the first age reported by other studies viz. 9 weeks (Kvac and Vitovec, 2003), 7 weeks (Wade *et al*., 2000) and 6 weeks (de J. Pena *et al*., 1997). A wider age range i.e. from neonates to adults has also been reported for *C. parvum* by some authors (Scott *et al*., 1995; Quilez *et al*., 1996; Ruest *et al*., 1998; Atwill *et al*., 1999), although most infected animals were pre-weaned calves. Also oocysts of both species have been observed in fecal samples from the same animal (Upton and Current, 1985). Therefore this necessitate that any study on *Cryptosporidium* in cattle should involve a diagnostic test that can precisely discriminate between the two species of *Cryptosporidium.*

In this study *C. andersoni* was not found to be associated with diarrhea ($P > 0.05$), although 7.44% of the diarrheic calves were observed to shed oocysts. The odds of shedding *C. andersoni*

was increased by 3.23 times among non-diarrheic calves when compared to the odds of shedding among diarrheic (20.6%: 7.44%). A similar finding was reported in calves of British Columbia (Olson *et al*., 1997a) and USA (Wade *et al*., 2000). It was also reported by Anderson (1982) that although at later ages calves appear to be more heavily infected, they do not show severe clinical symptoms.

As shown in Table 7, the frequency of cleaning calf rearing houses strongly influenced ($P < 0.001$) the detection of *Cryptosporidium* in dairy calves. Calves in poorly cleaned (monthly) farms were 5.2 times more likely to be infected with *C. andersoni* than calves in well-cleaned (daily) farms. Also calves in weekly-cleaned farms were 2.5 times more likely to be infected with *Cryptosporidium* than calves in daily-cleaned farms. These unhygienic farms were dirty and muddy, which could favor the persistence of *Cryptosporidium spp*. on the farms. Given the high concentration of oocysts that can be shed by infected dairy calves (Xiao and Herd, 1994; Nydam, 2003), poorly cleaned calf-houses would be a significant source of oocysts for calves.

Although small herd size farms had a significantly greater percentage of positive calves (23%) than had the large (14.3%) and medium (14.7%) sized farms in univariate screening analysis, this association was no longer observed in the multivariate model (Table 10). In further analysis of the questionnaire data, it was observed that out of the total farms regarded as poor hygienic, 94% were small herd-size farms of which 75 % were positive for *Cryptosporidium*. The effect of small herd size, therefore, resulted from the confounding influence of hygiene, and did not truly contribute to the risk of the disease. In several studies overseas, an association between large herd size and the risk of infection with *Cryptosporidium* has been observed, whereby increased density of animals favors infection of greater number of calves which in turn, contaminate their surroundings (Garber *et al*., 1994; Quigley *et al*., 1994; Mohammed *et al*., 1999).

Although some authors have observed an increase in the prevalence of cryptosporidiosis during certain seasons, related with high rainfall or the number of births (Garber *et al*., 1994; Atwill *et al*., 1999; Mohammed *et al*., 1999; Lefay *et al*., 2000), no significant association ($P > 0.05$) was observed between the season of the year when animals were sampled and the likelihood of infection with *Cryptosporidium*. This may partly be due to the minimal climatic variations seen

during the study period and more importantly to the resistance of the oocysts to adverse environmental conditions. Lack of seasonality of the infection has also been observed in other studies (Becher *et al*. 2004; Castro – Hermida *et al*., 2002; Wade *et al*., 2000; Ongerth and Stibbs, 1989). Similarly lack of significant association of the infection with geographic location from where samples were taken was observed, although it was significant in univariate analysis. This can also be related to the resistance of the oocysts to a wide range of environmental conditions.

As indicated in Table 7, there was a significant association (P = 0.003) between the feeding system of calves and the risk of infection with *Cryptosporidium*, where calves feeding directly on the ground were 2 times more likely to be infected with *Cryptosporidium* than calves feeding in feed troughs. However, this was not shown in multivariate analysis. Although preweaning calf housing condition of calves was found to be associated with the risk of infection in initial screening analysis, this was no longer observed in the multivariate analysis. The cleaning method of the floor of calf rearing houses had a significant association with *Cryptosporidium* infection where the odds of shedding the oocysts was increased by 1.6 times among farms practicing only sweeping when compared to farms practicing washing the floors. Therefore, washing the facilities appeared to be more effective in controlling *Cryptosporidium* contamination than was simply removing the manure.

Analysis of the findings with multiple (multivariate) logistic regression revealed that out of the several factors that had significant association with the risk of infection in initial screening analysis, only the frequency of cleaning calf rearing houses was significantly associated with the likelihood of infection with *Cryptosporidium*. Therefore, among the hypothesized factors, frequency of cleaning calf-rearing houses (hygiene) was the most important risk factor found to be responsible for the occurrence of *Cryptosporidium* infection on the farms.

5.2 *Eimeria* species

The overall point prevalence of *Eimeria spp*. infection (68.1%) is much higher than the two previous reports present in the country: 24.9% by Kassa *et al*. (1985) and 20% by Kebadu (1998). The present finding is also higher than reports from different countries: 49.6% in Poland (Pilarczyk *et al*., 2000), 46% in Netherlands (Cornelissen *et al*., 1995), 34.1% in Saudi Arabia (Kasim and Al-Shawa, 1985), 19.3-59% in Japan (Hasbullah *et al*., 1990; Oda and Nishida, 1990) and 29-52% in South Africa (Matjila and Penzhorn, 2002) but lower than reports from USA (86.3%) (Ernst *et al*., 1987) and Mexico (87.8%) (Rodriguez-Vivas *et al*., 1996). Comparable findings were also reported in some countries: 67.4% in a neighboring country, Kenya (Munyua and Ngotho, 1990), 68% in Turkey (Arslan and Tuzer, 1998), and 64.2% in Canada (Kennedy and Kralka, 1987).

There was a strongly significant association ($P < 0.001$) between the geographic zones samples were taken from and the risk of infection with *Eimeria spp*. The odds of shedding *Eimeria* oocysts was increased by 2.42 times among calves in Addis Ababa dairy farms when compared to the odds of shedding among calves in Debre Zeit dairy farms (Table 8). This is most likely attributed to differences in climatic conditions particularly rainfall and relative humidity of the two areas. Addis Ababa is located in a highland area at an altitude of 2500m and has got relatively higher mean annual rainfall of 1800mm and relative humidity of 60-80%, while Debre Zeit is located in midland at an altitude of 1850m and has a relatively lower rainfall of 800mm and relative humidity (50-60%) lower than that of Addis Ababa. This probably has created a more conducive climatic condition for the survival and sporulation of the oocysts in Addis Ababa than in Debre Zeit. Similar results have also been reported from other studies such as in Mexico (Rodriguez-Vivas, 1996) where higher infection rate was observed in high rainfall zones of the country.

The age of the calves also was strongly associated ($P<0.001$) with risk of infection where the prevalence *Eimeria spp* appeared to follow an age pattern with older calves showing higher rates of infection than younger calves. As shown in Table 8, higher infection rate was observed in calves 6-12 months of age (81%) than calves 0-6 months of age (61.4%) and the odds of

shedding *Eimeria* oocysts among calves 6-12 months of age was 2.7 times the odds of shedding among calves younger than 6 months. As observed during the study period, almost all of the calves older than 6 months were housed communally in overcrowded condition and in physical contact with adult animals, giving more chance for the animals for licking each other and thereby facilitating the transmission of *Eimeria* oocyst. The present findings are also consistent with those of other studies (Kennedy, 2001; Pilarczyk *et al*., 2000; Rodriguez-Vivas, 1996; Oda and Nishida, 1990).

Whether the calves fed directly on the ground or in feed troughs had a significant association (P=0.008) with the likelihood of *Eimeria* infection and the odds of shedding *Eimeria* oocysts was increased by 1.8 times among calves feeding directly on the ground when compared to the odds of shedding among calves feeding in troughs. Although this association was no longer observed in multivariate analysis, it is consistent with the recommendation in the literature of not feeding cattle especially calves on the ground because this increases the likelihood of contamination of the feed with manure containing *Eimeria* oocysts (Radostits *et al*., 1994; Kennedy, 2001).

In the present study a total of 11 species of *Eimeria* were identified based upon the characteristics of the oocysts of cattle as described by Levine (1985) and Soulsby (1982). This suggests that *Eimeria* species are abundant in Ethiopian dairy farms. Multiple infections in a single host with several *Eimeria spp.* were common findings in this study. The maximum number of species per sample ranged from 2 to 7 as shown in Table 6. This is within the same range as reported for calves in different countries: seven species were reported in USA (Ernst *et al*., 1987) and Netherlands (Cornelissen *et al*., 1995), six species in Turkey (Arslan and Tuzer, 1998) and five species in Canada (Kennedy and Kralka, 1987) and Turkey (Arslan and Tuzer, 1998).

Eimeria bovis (38.5%) was the most prevalent species investigated during the survey followed by *E. zuernii* (18.1%) and *E. auburnensis* (17.93%). This finding is consistent with most of the studies performed throughout the world and with what was described in literatures. Although a high proportion of calves were infected with the highly pathogenic *E. bovis* and/or *E. zuernii*, clinical coccidiosis was observed only in smaller proportion (16.20%) of calves positive for *Eimeria spp*. This finding is in agreement with Radostits *et al*. (1994) and a number of other

authors, who stated that most animals in a group become infected but only a minority (10-15%) develops clinical diseases. This is due to the normally low dose of oocysts ingested and subsequent development of immunity after a course of infection with a particular *Eimeria spp*. (Harper and Maas, 1996). Nevertheless, these subclinical infections might still negatively influence animal productivity and are even more important than clinical coccidiosis (Dedrickson, 2002) because they remain unrecognized by cattle producers and damage the absorptive surface of the intestine and weaken the immune system, leading to poorer feed efficiency, slow weight gain, weight loss, longer heifer development periods and increased susceptibility to other disease causing agents (Dairy herd management, 1998). Moreover, oocysts shed from these subclinically infected calves accumulate in the environment of calves or on the hair coats so that severe coccidiosis may develop when new calves are placed in these areas (Kennedy, 2001)

The mean (5109) and maximum (267000) oocyst excretion levels (OPG) observed in this survey were much higher than those reported from other countries. Kennedy and Kralka reported an average of 25 and a maximum of 109,449 OPG in Canada. Munyua and Ngotho (1990) reported a maximum of 30,600 OPG in Kenya. In Turkish study, a mean and maximum OPG of 1280 and 52000, respectively has been reported (Arslan and Tuzer, 1998). In the present survey, 10.4% of the infected calves had oocyst counts above 5000 OPG and out of these; the most pathogenic species, *E. bovis* and *E. zuernii* were encountered, respectively in 69.23% and 38.5% of the infected calves. A count of 5000 oocysts/g of feces is considered high enough by some investigators to warrant the judgment that the animal has the disease (Ernst and Benz, 1981; Rebhun, 1995). As indicated in Table 14, the mean OPG was significantly higher ($P = 0.023$) in diarrheic than non-diarrheic calves. This is inline with the hypotheses in literatures, which state that the development of clinical disease depends on the number of oocysts ingested (Kennedy, 2001; LAV, 1996).

Although the rate of infection increased with increasing age, the reverse was found to be true for the intensity of infection. As shown in Figure 4, the highest intensity of oocysts out put was observed among calves younger than 6 months and there was a sharp decline in OPG values as the age of calves increased. This implies that the immune system in younger calves is immature and consequently they are more vulnerable to coccidiosis but older calves are apparently able to

control infection or subsequent reinfections because of immunity they have developed from previous exposures. Similar results were also reported by some authors (Matjila and Penzhorn, 2002; Munyua and Ngotho, 1990).

The coccidia did not show a significant difference ($P > 0.05$) in infection rate or intensity of infection when compared with season of occurrence. Lack of seasonality may be related to the short duration of the study period and more likely to less climatic variations observed during the seven months of study based on the metrological data obtained from NMA. These findings are similar with that of other studies (Kennedy and Kralka, 1987; Adams and Perth, 2004). As reported by Adams and Perth (2004), the disease can be a problem at any time of year so long as conditions of adequate moisture and temperature exist for survival and development of oocysts.

6. CONCLUSION AND RECOMMENDATIONS

This study has clearly demonstrated that both *Cryptosporidium* and *Eimeria* species are prevalent in Central Ethiopian dairy farms. Based on oocyst morphology and epidemiological features, the species of *Cryptosporidium* circulating in the dairy farms surveyed was presumed to be *C. andersoni.* With regard to *Eimeria spp*. a total of 11 species were identified of which *E. bovis* was the most prevalent followed by *E. zuernii* and *E. auburnensis,* and *E. bukidnonensis* and *E. brasilensis* were the least prevalent species encountered.

In the present study, *C. andersoni* infection was detected at a higher rate in non-diarrheic than diarrheic calves and was not found to be statistically associated with any state of clinical manifestations and therefore, this agent is considered not to be a significant pathogen. However, since losses in weight gain and milk production has been reported by some studies, the detection and local awareness of the parasite as well as institution of preventive measures remains important. *C. parvum,* which is more pathogenic, was not detected in any of *Cryptosporidium*-positive calves even in the most critical age group. Nevertheless, this species is one of the most important causes of calf diarrhea in most parts of the world second only to rotavirus and the most frequently reported species in prevalence studies focused on *Cryptosporidium.*

In spite of the fact that most of the calves sampled were found to be infected with *Eimeria spp*., clinical coccidiosis was observed only in a small proportion of the infected calves. Therefore, it is concluded that most *Eimeria* infections in calves on Central Ethiopian dairy farms, even with *E. bovis* and *E.zuernii*, result in subclinical infections. Taking in to account the high proportion of the pathogenic spp, *E. bovis* and *E.zuernii* in infected calves; however, the subclinical infections they cause should not be underestimated as these infections can still negatively influence animal productivity and cause economic losses due to poorer feed efficiency, slow weight gain, weight loss, failure of the calves to grow to their full potential and increased susceptibility to other diseases.

In the final analysis, this study made it possible to identify three factors that were significantly associated with the risk of infection with *Cryptosporidium* and *Eimeria* species. The frequency of cleaning calf rearing houses was the most important risk factor found to be associated with *Cryptosporidium* infection, where the odds of detecting *Cryptosporidium* oocysts appeared to be high among calves in poorly cleaned farms. On the other hand, the geographical zone where samples were taken from and age of the calves were the most important risk factors that strongly influenced the detection of *Eimeria* oocysts in the dairy calves. The present observations indicate that *Cryptosporidium* infection in bovine due to *C. andersoni* can be observed at any age in calves but *Eimeria* infections are more important in calves above six months of age.

Therefore, based on these findings the following recommendations are forwarded that might help in preventing losses associated with the occurrence of the parasites in the dairy farms and thereby improving the productivity of the animals.

- Optimum hygienic conditions should be maintained on the farms including frequent removal of manure, washing and disinfecting the floor of calf rearing houses and hot water cleaning of feed and water utensils as this is the mainstay for the control of *Cryptosporidium* infection in calves

- Feeding of calves directly on the ground should be avoided as this may result in contamination of the feed with the oocysts of the parasites in manure.

- As older animals can serve as source of infection for the younger ones, keeping of calves in close contact with adult animals and also mixing of calves of different ages and size should be avoided.

- The calves should be watched regularly and those diarrheic calves must be isolated from the healthy ones and receive appropriate treatment.

- As calves were found to be infected by *Eimeria* spp as early as 15 days of life, the use of coccidiostats in ration is advisable to prevent calves from *Eimeria* infections.

- Molecular characterization is required to confirm the species of *Cryptosporidium* circulating in the farms as the present identification was based on conventional laboratory techniques and also a further study is needed to verify the absence of the pathogenic species, *C. parvum* from Ethiopian dairy farms

- Since the present study was a cross-sectional one that depicts only a momentary picture of the infection status of the herds, further longitudinal studies with repeated samplings are needed to give a more accurate assessment of the prevalence and economic impact of the parasites.

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8. ANNEXES

Annex 1. The morphology, size, sporulation time and pathogenecity of the commonly known *Eimeria* species of cattle.

Adapted from Soulsby (1982) and Levine (1985).

Eimeria species	Morphology of the oocysts	Size	Micropyle	Sporulation Time	Pathogenecity
1.*E. alabamensis*	Predominantly pear-shaped, some ellipsoidal, subcylindrical or asymmetrical Oocyst wall thin, homogenous, transparent and generally colorless	18.9μm x 13.4μm (13-24μm x 11-16μm)	Absent	96-120 hrs	Low under field conditions It is unusual in that the developmental stages occur in the nucleus of the epithelial cell.
2.*E. auburnensis*	Ovoidal, varying from ellipsoidal to tapering Oocyst wall smooth and homogenous, transparent, yellowish-brown in color; occasionally it may have a coarsely granulated surface and at times be heavily mammilated	38.4 μm x 23.1μm (32-46μm x 20-25)	Present	48-72 hrs	Low under field condition
3. *E. bovis*	Ovoidal, blunted across the narrow end; in massive infection a variation in shape may occur. Oocyst wall smooth, homogenous, transparent, greenish-brown in color.	27.7 x20.3 μm (23-34 μm x 17-23μm)	Present	48-72 hrs	Highly pathogenic

Annex 1. Continued

Eimeria species	Morphology of the oocysts	Size	Micropyle	Sporulation Time	Pathogenecity
4.*E. brasilensis*	Oval, oocyst wall colorless to yellow, smooth with a distinct polar cap	37.5 x 27.1 μm (34.2-42.7μm x24.2-29.9μm)	Present	6-7 days	Not pathogenic and the developmental cycle is unknown
5.*E.bukidnonensis*	Pear-shaped to oval, yellowish brown to dark brown in color. Oocyst wall shows radial striation.	44μm x 31.1 μm	Present	17 days	The developmental cycle is unknown Diarrhea has been observed in an experimentally infected calf
6.*E. canadensis*	Ellipsoidal, occasionally cylindrical; oocyst wall, smooth, transparent, slightly yellowish brown in color	32.5μm x 23.4μm (28.37μm x 20-27μm)	Present	72-96 hrs	The developmental cycle is unknown, as is the pathogenecity
7. *E. cylindrica*	Cylindrical, some may be narrow cylinders; oocyst wall, thin, colorless, smooth.	23.3μm x 13.3μm (16-27μm x 12-15μm)	Absent	2 days	The developmental cycle is not known

Annex 1 continued

Eimeria species	Morphology of the oocysts	Size	Micropyle	Sporulation Time	Pathogenecity
8. *E. ellipsoidalis*	Ellipsoidal, occasionally spherical or cylindrical; oocyst wall, thin, homogenous, and transparent	16.9μm x13μm (12-27μm x 0-18μm)	Absent	48-72 hrs	Have been reported causing diarrhea
9. *E.subspherica*	Smallest of all the bovine *Eimeria* spp. Oocyst wall, uniformly thin, smooth and transparent.	11μm x 10.4μm (9-11μm x 8-12μm)	Absent	4-5 days	Not pathogenic The developmental cycle is unknown.
10. *E. zuernii*	Spherical, sub-spherical to ellipsoidal oocyst wall, thin, homogenous, transparent, colorless to pale yellow.	17.8μm x 15.6μm (15-22μm x 13-18μm)	Absent	9-10 days at12^{o}C 3 days at 20^{o}C 23-24 hrs at 30-32.5^{o}C	The major pathogenic
11.*E.wyomingensis*	Similar to *E. bukidnonensis* but the oocysts are slightly smaller and ovoidal. Oocyst wall, yellowish-brown to greenish-brown, slightly speckled.	40.3um x 28.1um (37-44.9um x 26.4-30.8um)	Present	5 to 7 days	The developmental cycle and the pathogenecity are unknown

Annex 2. Sample and laboratory findings record sheet

Study site	Name of the farm	Date of sampling	Calf Id. No	Breed	Sex	Age in days	Fecal consistency	Parasites identified On flotation	OPG	Acid-fast test	Remarks

Annex 3. List of laboratory reagents

3.1. Reagents for Sheather's sugar preparation

Granulated sugar (sucrose): 454gm
Tap water: 355ml
Formaldehyde (40%): 6ml

3.2 Reagents for Kinyoun acid-fast stain

For preparation of carbol fuchsine
Basic fuchsine: 4gm
Phenol (melted crystal): 8ml
Ethanol (95%): 20ml
Distilled water: 100ml

For preparation of docolorizer
Ethanol (95%): 97ml
Hydrochloric acid (concentrated): 3ml

For preparation of counter stain
Methylene blue: 0.3gm
Distilled water: 100ml

Annex 4. Questionnaire for collecting data on various risk factors associated with protozoal causes of calf diarrhea in dairy farms

Date----------

Farm owner----------

Business name of the farm----------

Farm code----------

Address----------

---

1. Herd size, composition and breed:

1.1 Cows: exotic_____cross_____local____Pregnant cows expected to calve soon____

1.2 Heifers: exotic_____cross_____local____

1.3 Oxen:_exotic_____cross_____local_____

1.4 Bulls:_exotic_____cross_____local_____

1.5 Male calves:

0 - <6monthes_____exotic_____cross____local_____

6m -<1year_____exotic_____ cross_ ___local_____

1.6 Female calves:

0 - <6monthes_____exotic_____cross_____local____

6m - <1year_____exotic_____cross_____local_____

1. Calf housing

2.1 Pre weaning

Group pen in calf barn: _____

Group pen in cow barn_____

Individual pen in calf barn: _____

Individual pen in cow barn:_____

Tethered along an inside wall of the main barn_____

Outdoor grouped in pen_____

Outdoor in individual pen_____

2.2. Post weaning

Group pen in calf barn: _____

Group pen in cow barn_____

Individual pen in calf barn: _____

Individual pen in cow barn: _____

Tethered along an inside wall of the main barn_____

Outdoor grouped in pen_____

Outdoor in individual pen_____

2.3. Mixing of calves with different age and size

Yes_____

No_____

2.4 Type of floor in the barn (Earth, metal, concrete, wood)_____

2.5 Bedding in calf pen (litter, no)_____

3. Sharing of facilities with adult animals

Yes_____

No_____

4. Hygienic conditions:

4.1 Frequency of cleaning calf barns

Daily_____

Weekly_____

Every 2 weeks_____

4.2 Method of cleaning

Dry cleaning (sweeping) only_____

Changing of litter bedding_____

Washing _____

Washing and disinfections_____

4.3. Drainage system

Yes_____

No_____

5. Maternity facilities:

Multiple cow_____

Individual cow_____

No_____

6. Do you practice an all-in / all-out management system

Yes_____

No_____

7. Milk feeding

7.1.How soon is colostrum fed to the newborn?

Immediately after birth (< 6 hrs)_____

Between 6 to 12 hrs_____

Between 13 to 24 hrs_____

After 24 hrs_____

7.2. When are calves separated from dams?

Immediately before nursing_____

Immediately after nursing_____

2-12 hr after birth_____

13-24 hr after birth_____

7.3. Method of milk feeding

Natural sucking by the calf_____

Feeding with a nipple_____

Tube feeding_____

Bucket feeding_____

7.4. Do you think colostrum is good for newborn calves (yes/no)? _____

7.5. Do you restrict the newborns from ingesting colostrum (yes/no)? _____

8. Feeding and provision of water

8.1.Supplemental feeds to calves from birth to weaning (yes/no)? _____

8.2. What type of feed (if yes)?

Commercial_____

Home made concentrates_____

8.3. Do you feed calves milk replacers (yes/no)? _____

8.4. Feeding system

In feed troughs_____

On the ground_____

8.5. Are feeding troughs separate for each calf or common? _____

8.6.Source of water for calves (river, well, tap, etc)_____

8.7.Watering utensils

Separate_____

Common_____

9.Weaning age calves in the farm:

a) Before 5 weeks_____
b) Between 5to 8 weeks_____
c) Between 8 to 12 weeks_____
d) After 12 weeks_____

10. Health management of calves in the farm:

10.1.What health problems do your calves' encounter frequently?

Pneumonia_____

Diarrhea_____

Navel ill_____

Unthriftiness_____

Early mortality_____

Others (specify)_____

10.2. If diarrhea occurred in calves last year, what treatment was given?

Rehydration fluid_____

Antibiotics_____

Anthelminthics_____

Antiprotozoals_____

10.3.What do you think the main cause of calf diarrhea in your farm?

Parasitic_____

Bacterial_____

Viral_____

Others (specify)_____

10.4.Do you feed your calves coccidiostats (yes/no)? _____

10.5.Is there regular deworming of calves in your farm (yes/no)? _____

11. Where do replacement stock come from?

a) Entirely home reared_____

b) Purchased_____

12. Who is in charge of feeding and caring for calves in your farm?

a) Owner himself_____

b) Owner's wife_____

c) Hired labor_____

Annex 5. Results of questionnaire survey

Sr. No	Factor	N	Percentage (%)	Remarks
1	**Herd size**			
	Small	30	75	<50
	Medium	7	17.5	50-150
	Large	3	7.5	>150
2	**Herd composition**			
	Cows	1170		
	Heifers	469		
	Bulls	69		
	Calves <1year	580		
3	**Preweaning housing condition**			
	Individual pen	11	27.5	
	Group pen	16	40	
	Tethered in cow/calf barn	11	27.5	
4	**Post weaning housing condition**			
	Individual pen	20	50	
	Group pen	20	50	
5	**Frequency of cleaning calf rearing house**			
	Daily	27	67.5	
	Weekly	10	25	
	Monthly	3	7.5	
6	**Method of cleaning**			
	Sweeping/Scraping feces	26	65	
	Sweeping and washing	14	35	
7	**Drainage system**			
	Yes	21	52.5	
	No	19	47.5	

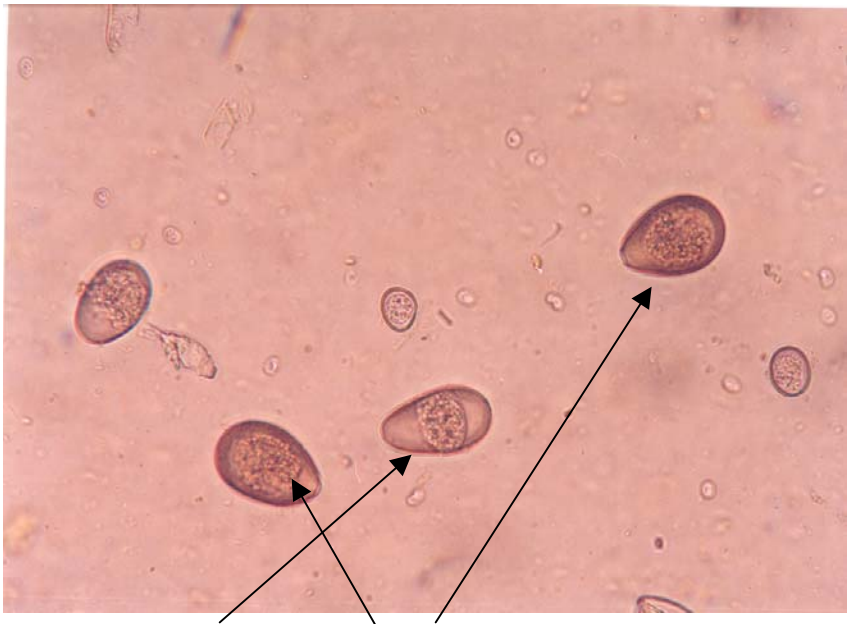
Annex 5 continued

Sr. No	Factor	No	Percentage (%)	Remarks
8	**Use of bedding**			
	Yes	14	35	
	No	26	65	
9	**Maternity pen**			
	No	28	70	
	Individual pen	2	5	
	Multiple	10	25	
10	**Milk feeding system**			
	Isolated immediately and bucket fed	33	82.5	
	Isolated after colostrum period and bucket fed	5	12.5	
	Natural suckling by the calf	2	5	
11	**Feeding system of calves**			
	In feed troughs	22	55	
	On the ground	18	45	
12	**Watering utensils**			
	Common	32	80	
	Individual	8	20	
	Source of water			
	Tap	35	87.5	
	Well	2	5	
	Spring	1	2.5	
	River	1	2.5	
13	**Out door exercise**			
	Yes	16	40	
	No	24	60	
14	**Proximity of calves to cows**			
	Yes	27	67.5	
	No	13	32.5	
15	**Use of coccidiostats**			
	Yes	40	100	
	No	0	0	
16	**Common health problems**			
	Diarrhea	20	50	
	Early calf mortality	3	7.5	
	Unthriftness	7	17.5	
	Pneumonia	3	7.5	
	Bloat	1	2.5	
	Joint ill	1	2.5	

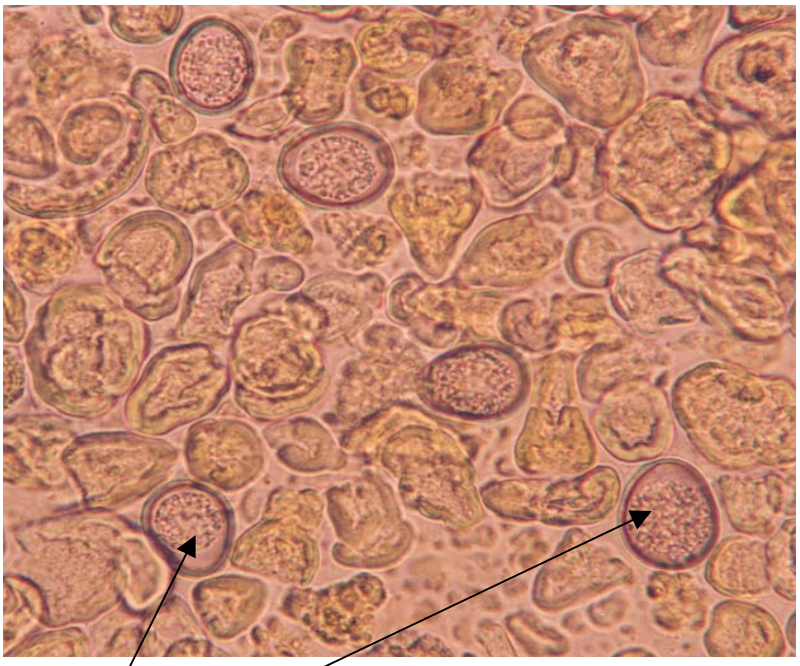
Annex 6. Analysis of the association between season of the year and OPG of *Eimeria spp.* with One-way ANOVA

Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1372653021.247	2	686326510.624	1.531	0.218
Within Groups	1372653021.247	2	686326510.624		
Total	112135428088.400	249			

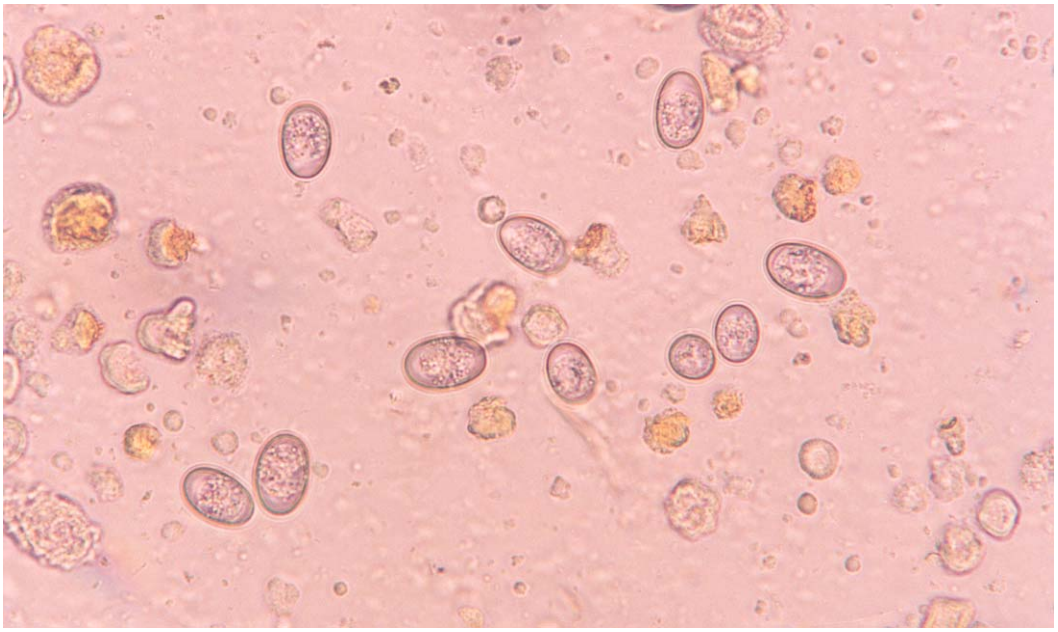
Annex 7. Pictures of some of the different species of *Eimeria* identified during the study period



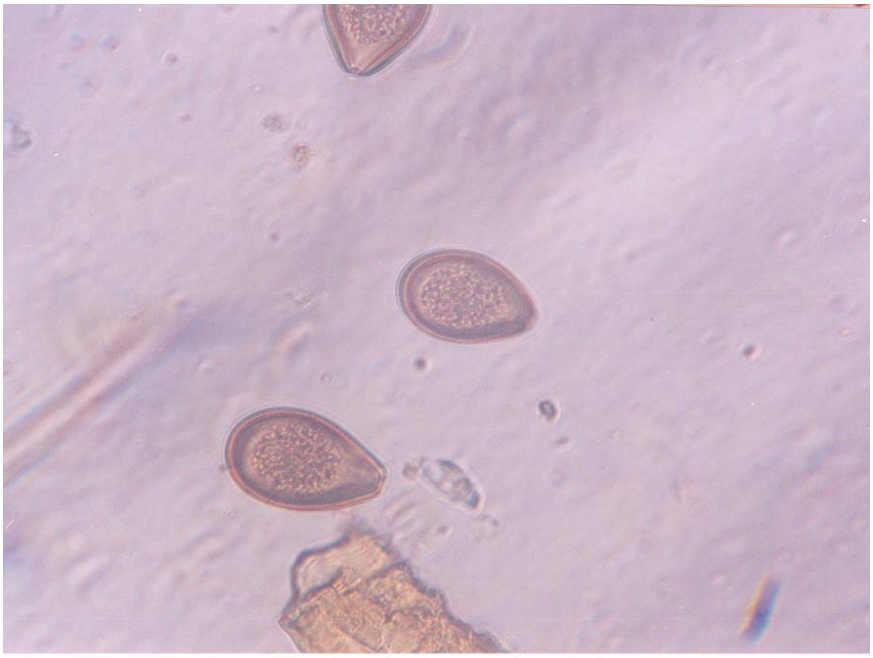
a) *E. auburnensis* and *E. wyomingensis*

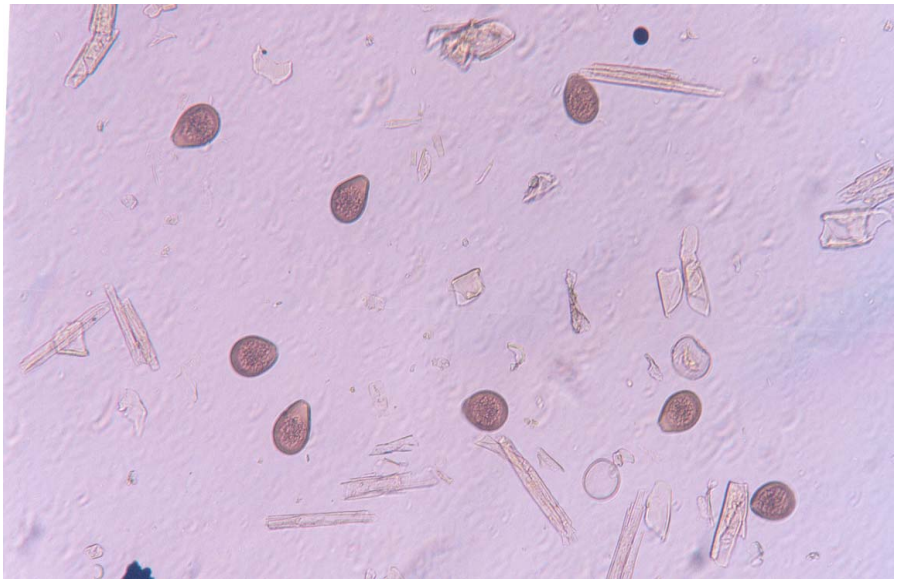


b**).** *E. bovis*

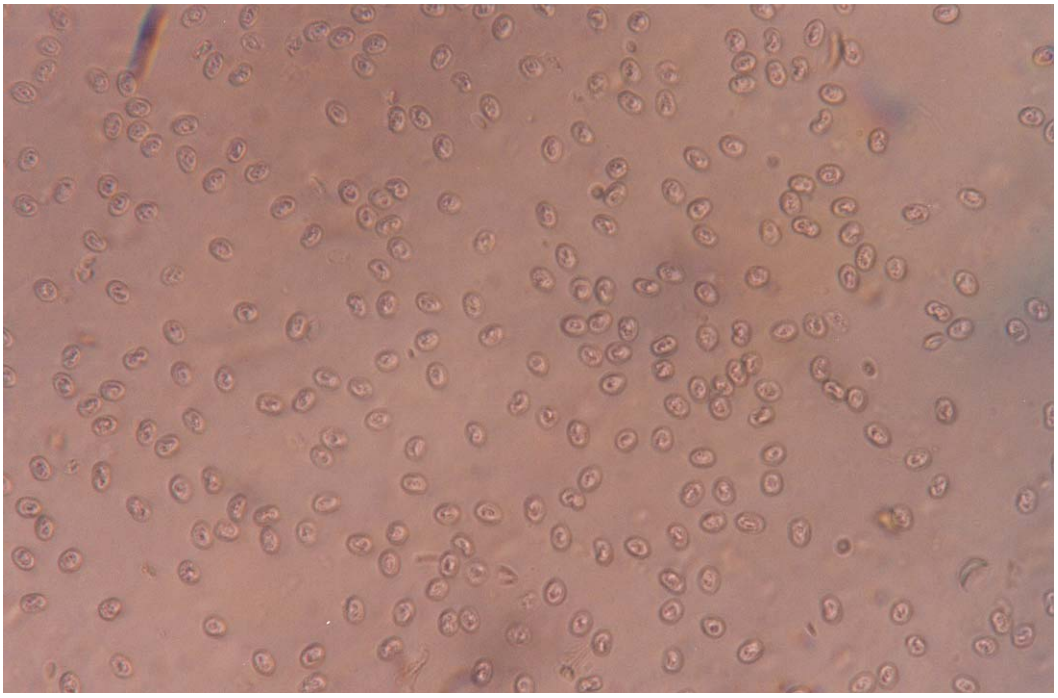


c) *E. ellipsoidalis*

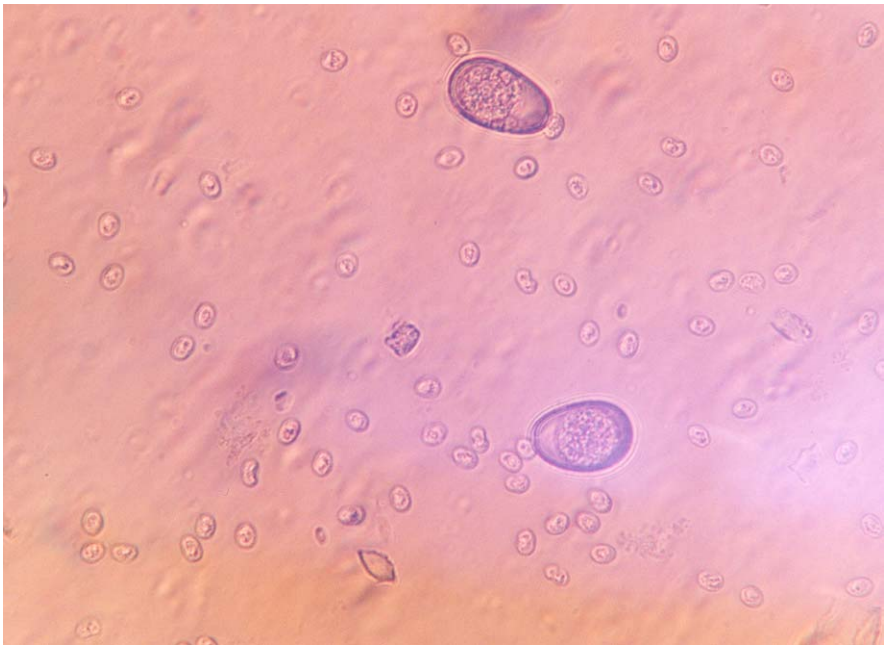




d) E. bukidnonensis



e) *Cryptosporidium andersoni* (x400)



f) Mixed infection of *Cryptosporidium* and *Eimeria* spp

Annex 8. Point prevalence of *Cryptosporidium* and *Eimeria* infection at farm level

Sr. No	**Name of the farm**	**Location**	**Herd size**	**No. calves sampled**	**%Pos.*Crypto sporidium***	**%Pos. *Eimeria* spp**
1	Adil Muktar	Addis Ababa	Small	10	30.00	80.00
2	Almaz	Debre Zeit	Small	8	0.00	62.50
3	Amede Lema	Addis Ababa	Small	7	28.57	42.86
4	Antonio Valentina	Addis Ababa	Small	7	0.00	71.43
5	Asefa G/Michael	Addis Ababa	Small	7	0.00	100.00
6	Asegedech Degefu	Addis Ababa	Small	13	23.08	53.85
7	Ashenafi Asnakew	Addis Ababa	Small	3	33.33	33.33
8	Bezunesh	Addis Ababa	Small	4	50.00	100.00
9	Boni Agro industry	Addis Ababa	Small	9	0.00	77.78
10	D.Z.A.R.C	Debre Zeit	Medium	13	7.69	46.15
11	D.Z.D.F	Debre Zeit	Medium	34	17.65	50.00
12	Debebe	Addis Ababa	Large	50	16.00	84.00
13	Dibaba Hurrissa	Addis Ababa	Small	3	0.00	33.33
14	Edeget Kebede	Addis Ababa	Small	4	0.00	50.00
15	Eleni Azene	Addis Ababa	Small	17	23.53	76.47
16	Eshete Yigzaw	Addis Ababa	Large	47	14.89	76.60
17	Genesis	Debre Zeit	Medium	22	0.00	36.36
18	Haji Nuru	Addis Ababa	Small	10	60.00	100.00
19	ILRI	Debre Zeit	Large	134	13.43	63.43
20	Jemanesh Jimma	Addis Ababa	Small	3	0.00	100.00
21	K.H.C.d.f.	Debre Zeit	small	20	45.00	75.00

22	Kassa Erketa	Addis Ababa	Small	2	0.00	100.00
23	Kelemework W/Amanuel	Addis Ababa	Medium	20	5.00	80.00
24	Legesse Tefera	Addis Ababa	Small	9	11.11	88.89
25	Meles Lakew	Addis Ababa	Small	5	0.00	80.00
26	Moges H/Silase	Addis Ababa	Small	6	50.00	66.67
27	Nuru Mohammed	Addis Ababa	Small	4	25.00	75.00
28	Repi PLC	Addis Ababa	Medium	22	31.82	77.27
29	Seada Ali	Addis Ababa	Medium	6	66.67	100.00
30	Senay Beyene	Addis Ababa	Small	5	0.00	60.00
31	Sharew Hussein	Addis Ababa	Small	2	100.00	50.00
32	St. Khilara church	Addis Ababa	Small	10	0.00	60.00
33	Tamirat Ali	Addis Ababa	Small	4	75.00	75.00
34	Tefera Mulat	Addis Ababa	Small	9	11.11	66.67
35	Tesfaye Damtew	Addis Ababa	Small	10	20.00	60.00
36	Thomas Matanovich	Addis Ababa	Small	6	0.00	66.67
37	Tseday	Debre Zeit	Medium	19	5.26	36.84
38	W/Kidane Nerie	Addis Ababa	Small	7	42.86	85.71
39	Woynishet G/Medehin	Addis Ababa	Small	4	0.00	100.00
40	Zelalem Fisseha	Addis Ababa	Small	5	60.00	80.00

9. CURRICULUM VITAE

Personal Information

Name: Rahmeto Abebe Basher

Date of birth: December 25, 1969

Place of birth: Hosanna, Hadiya Zone, SNNPRS

Marital status: Single

Language skill: Amharic, Siltigna, Hadiyigna and English

Nationality: Ethiopian

Contact Address: rahmetobeshir@yahoo.com

Educational background:

Period	Institution	Award
1974-1982	Yekatit 25/67 Junior and secondary school	Certificate
1982-1986	Wachamo comprehensive secondary school	Certificate
1986-1992	Faculty of Veterinary Medicine, Addis Ababa University	DVM degree
2003-2005	Faculty of Veterinary Medicine, Addis Ababa University	MSC degree

Trainings:

Participatory Rural Appraisal

Communication and Social mobilization

Computer- word processor, spreadsheet, and database management

Work Experience:

March 1994 – August 2000: SNNPRS, Hadiya zone, Badawacho woreda Agricultural office veterinary services tea leader

September 2000 – September 2001: SNNPRS, Hadiya zone, Misha woreda Agricultural office veterinary services tea leader

October 2001 – June 2003: SNNPRS, Siltie zone rural development coordination main department, veterinary services team leader

July 2003 – to date: SNNPRS, Siltie zone rural development coordination main department, Agricultural and natural resources development desk head

Major Additional Responsibilities:

- Coordinator of UNICEF- assisted Woreda Integrated Basic Services (WIBS) program for 4 years
- Planning and Project team leader of local development association for 6 months

Research out puts:

Rahmeto Abebe, 1992. Faciolosis: - Clinical occurrences, Coprological, Abattoir, and Snail survey in and around Wolisso. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University

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Review on Major Parasitic Causes of Calf Diarrhea Seminar on current topics, March 2004, Addis Ababa University Faculty of Veterinary Medicine, Debre Zeit

Membership of Scientific Society:

- Ethiopian Veterinary Association

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Ato Usman Surur, Head of Siltie zone Rural Development Coordination Main Department, SNNPRS

10. SIGNED DECLARATION SHEET

I the undersigned, declare that this thesis is my original work, has not been presented for a degree in any other University and that all sources of material used for the thesis have been dully acknowledged.

Name: Rahmeto Abebe Beshir

Signature: _____

Date of Submission: _____

This thesis has been submitted for examination with my approval as an academic advisor.

Dr. Abebe Wossene_____