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ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

STUDIES ON COCCIDIOSIS AND CRYPTOSPORIDIOSIS IN SMALL RUMINANTS AT ELFORA EXPORT ABATTIOR AND IN AND AROUND DEBRE -ZEIT, ETHIOPIA

DINKA AYANA AGA

June 2006
Debre-zeit, Ethiopia

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STUDIES ON COCCIDIOSIS AND CRYPTOSPORIDIOSIS IN SMALL RUMINANTS AT ELFORA EXPORT ABATTIOR AND IN AND AROUND DEBRE-ZEIT, ETHIOPIA

A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary Epidemiology

BY

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Board of Examiners: Signatures

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LIST OF ABBERVIATIONS

AAU: Addis Ababa University

AIDS: Acquired Immune Deficiency Syndrome

CI: Confidence Interval

ELISA: Enzyme Linked Immunosorbent Assay

FAT: Fluorescent Antibody Test

FVM: Faculty of Veterinary Medicine

i.e.: That means

IF: Immunoflourescence

LAV: Large Animal Veterinarian

OPG: Oocysts Per Gram of faeces

PCR: Polymerase Chain Reaction

Spp.: Species

SPSS: Statistical Packages for Social Science

χ^2: Chi-square

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ABSTRACT

A cross sectional study on cocciodiosis and cryptosporidiosis in small ruminants at ELFORA export abattoir and in & around Debre-zeit was conducted from October, 2005 to February, 2006. In this study, attempts were made to determine the prevalence and intensity of *Eimeria* and *Cryptosporidium* infection in sheep and goats. More over, potential risk factors associated with the infections and the species of *Eimeria* and *Cryptosporidium* incriminated in the infections were identified. A total of 1152 faecal samples (384 from animals intended for slaughter at ELFORA export abattoir and 768 samples from those reared under extensive condition of Debre-zeit and its surrounding areas) were collected and examined by faecal flotation using concentrated sugar solution (Sheather's sugar solution) to detect the oocysts of *Eimeria* and *Cryptosporiudium* species. Faecal samples suspected of *Cryptosporidium* were also examined under a microscope using modified Kinyoun acid-fast staining technique. Measurement (Micrometery) of oocysts to identify the species involved in the infections and determination of oocysts per gram of faeces (OPG) were also conducted. Out of the 1152 faecal samples collected and examined, 346(49%) ovine and 173(38.5%) caprine were found to be infected with *Eimeria* species. Only four (0.57%) faecal samples collected from sheep were infected with *Cryptosporidium andersoni*. At Debre-Zeit and its surroundings, 37.8% and 0.5% prevalence of *Eimeria* and *Cryptosporidium* infections were respectively registered. While 59.6% and 0 prevalence of *Eimeria* and *Cryptosporidium* infections were recorded respectively at ELFORA. Statistically, a significant variation ($P<0.001$) in the prevalence rate of *Eimeria* infection between the study areas, between animal hosts, among the months of the study period and between different age groups was observed. However, there was no significant difference ($P>0.05$) observed in the prevalence rate of *Eimeria* infection between the two sexes of the study animals. There was no significant variation ($P>0.05$) observed in the prevalence of *Cryptosporidium* infection between the two study sites, animal hosts, animal sex, between age groups and among the months of the study period. There was no significant variation ($P>0.05$) observed in the mean OPG values of *Eimeria* between the

two age groups of the study animals. High and low mean OPG value of *Eimeria* oocysts in November and December were respectively recorded.

KEY WORDS: ***Coccidiosis/ Cryptosporidiosis /Small ruminants/ELFORA export abattoir/ Debre-Zeit & its surroundings***

1. INTRODUCTION

Africa has a high population of 205 million sheep and 174 million goats representing approximately 17% and 31% of the world total small ruminant population respectively (FAO, 1990). The population of sheep in sub-Saharan Africa is estimated at 127 million head, while that of goats is estimated to be 147 million (Winrock, 1992). Indigenous sheep and goat breeds constitute over 95% of the small ruminant population of Africa. They are owned by the majority of smallholder rural farmers for whom this resource is critical for nutrition and source of income. Sheep and goats are highly adapted to broad range of environments and can utilize a wide variety of plant species and are thus complementary to cattle and camel production (Rege, 1992).

Ethiopia with its great variation in climate and topography possesses one of the largest livestock populations in the world. It is estimated that 35.1 million cattle, 12.2 million sheep, 9.5 million goats, more than 4.5 million equines and 30.1 million poultry are found in this country. Of the total sheep population in Ethiopia, 75% are raised in the high lands with altitude of above 1500m a.s.l. sustaining 92% of the human population. The rest 25% are reared in the low lands. Goats are widely distributed in all climatic zones but with a high concentration in dry areas (ELSMA, 1999).

In spite of the huge small ruminant population in Ethiopia however, under developed infrastructure coupled with poor management practices, low nutritional status, poor genetic makeup and diseases considerably affect the productivity of this sub sector. The share of parasitic diseases in this regard has been of paramount importance. Losses from livestock production due to parasitic diseases are very high both in developed and developing countries (Agemang *et al.*, 1995). Among parasitic diseases, coccidiosis due to different species and cryptosporidiosis due mainly to *Cryptosporidium parvum* are important protozoal diseases responsible for low productivity and mortality in small ruminants especially in young ones. Coccidiosis is a parasitic disease caused by an

intracellular protozoa in vertebrates and invertebrates. The disease is of economic importance affecting birds, cattle, sheep, goats and other domestic animals (Levine, 1985). In sheep and goats, coccidiosis is caused by parasites of the genus *Eimeria* and is an important disease especially in pre-weaned and recently weaned lambs and goat kids. While nearly all animals are exposed to *Coccidia*, they may not show signs of disease (Pout *et al.*, 1973). This condition known as sub clinical coccidiosis has a significant impact on the economics of animal production, causing a reduction in weight gain and feed efficiency and increased susceptibility to other diseases. Clinical coccidiosis results in even higher financial losses to producers because of medical treatment costs, a more sever effect on growth performance and sometimes death losses.

Clinical coccidiosis in domestic animals became an economically important problem with the introduction of intensive rearing systems. Diseases out breaks were associated with high stocking density, very poor weather conditions and use of restricted areas to supplement the flock with extra food (Deam and Throp, 1939). Lambs in lambing pens, intensive grazing areas and feedlots are at great risks of coccidiosis as a result of shipping, ration change, crowding stress, severe weather and contamination of the environment with oocysts from ewes or other lambs. Kids appear to be much more susceptible to coccidiosis acquiring the infection through ingestion of sporulated oocysts of *Eimeria* species (Urquhart *et al.*, 1996).

Cryptosporidiosis, caused by the genus *Cryptosporidium*, is another important protozoal disease of young animals and humans with a cosmopolitan distribution. Contrary to coccidiosis, the disease is not host specific and affects several species of animals (Urquhart *et al.*, 1996). In addition, the parasite lacks organ and host specificity and is resistant to various anti microbial agents. *Cryptosporidium* has the ability for autoinfection and occupies a very unique location within the host cell membrane (Tizipori *et al.*, 1983).

As to the distribution of these infections, several reports were made from different countries. For instance, Majewska *et al.* (2000), reported a 10.1% prevalence of

Cryptosporidium species in sheep in West-central region of Poland; 98% infection rate of *Coccidia* in populations of goats in Australia and 20-40% mortality rate of coccidiosis among kids on different farms in Zimbabwe have been documented (Radostitis *et al.*, 1994). The diseases are also important in other species of ruminants. In the USA, 50% of dairy calves shed oocysts of *Cryptosporidium* and the parasite is present on more than 90% of dairy farms in the continent (Anderson and Hall, 1982). In a study conducted in North–West Poland, Pilarczyk and Balicka-Ramsiz (2000), reported 27% and 49.6% infection rates of coccidiosis in dairy cows and calves, respectively. In Ethiopia, Kebadu (1998), reported a 20% prevalence of *Eimeria bovis* in calves in Debre Zeit smallholder dairy farms.

Worldwide, the prevalence of cryptosporidiosis in humans is 1 to 4.5% in developed countries and 3 to 20% in developing countries. Estimated infection rates in AIDS patients range from 3 to 20% in the United States and 50 to 60% in Africa (Juranek, 1994).

In Ethiopia, though some works have been conducted to determine the prevalence and economic significance of coccidiosis in poultry and calves, there is no documented report of coccidiosis and cryptosporidiosis in small ruminants. These might partly be due to the inadequacy of sophisticated diagnostic facilities especially for the diagnosis of *Cryptosporidium* species, and partly due to the underestimation of the significance of these diseases in small ruminants by animal health professionals. Therefore, the objectives of this study are:

1. To determine the prevalence and intensity of *Eimeria* and *Cryptosporidium* infections in sheep and goats.
2. To identify the species of *Eimeria* and *Cryptosporidium* incriminated in the infections.
3. To identify factors influencing these infections.

2. LITREATURE REVIEW

2.1 Historical perspectives

Coccidiosis as a distinct clinical disease of cattle was described by Zurn in 1878 (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 parasite stages that he saw and hence present- day investigators cannot distinguish that species from the other species, which are now recognized from cattle.

Cryptosporidium was first described by Tyzzer (1907), when he isolated the type species, which he named *Cryptosporidium muris*, from the gastric glands of laboratory mice. He latter 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 containing 4 sporozoites not enclosed within secondary spores (sporocysts). Tyzzer (1912) found a second isolate, which he named *Cryptosporidium parvum*, in the small intestine of the same species of laboratory mice but they were smaller in size than *Cryptosporidium 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. From 1983 onwards, with the onset of the AIDS epidemic, *Cryptosporidium* emerged as a life threatening disease in humans. In

1993, it reached the public domain when it became widely recognized as the most serious and difficult to control, cause of water born related diarrhea (Mackenzie *et al.*, 1994). The first glimpse of the seriousness of *Cryptosporidium* in mammals, mainly calves were provided in the late 1970s. Until then, *Cryptosporidium* was mostly identified histologically in infected gut sections or in biopsy specimens and was considered to be an opportunistic protozoan that caused a few or no symptoms. At present, *Cryptosporidium* infection is a well-recognized cause of diarrhea in immunocompitant and immunocompromised humans and other animals of agricultural interest through out the world (Abrahamsen, 1998). Only *Cryptosporidium parvum* is known to be associated with diarrhea in neonatal ruminants (de Graaf *et al.*, 1999).

2.2 Taxonomy/classification

Taxonomically, *Cryptosporidium* belongs to the Phylum Apicomplexa (which posses an apical complex), Class Sporozoasida (which reproduce by asexual and sexual cycles, with oocyst formation), Sub class Coccidiasina (with a life cycle involving merogony, gametogony and sporogony), Order Eucoccidiida (in which schizogony occurs), sub order Eimeriina (in which independent micro and macrogamy develop), Family Cryptosporiidae (contain four naked sporozoites within oocysts but no sporocysts) (Tzipori and Ward, 2002).The same classification holds true for *Eimeria* except that they belong to a different family, Eimeriidae and the Genus *Eimeria* (Upton, 2003). Members of the family Cryptosporididae are also homoxenous, but they differ from the Eimeridae in that they develop just under the surface membrane of the host cell or within its brush boarder rather than in the cell proper. In addition, their meronts have a knoblike attachment organelle, and the microgametes lack flagella (Levine, 1985).

2.3 Basic biology and characteristics of *Cryptosporidium parvum*

Many aspects of the biology and nature of *Cryptosporidium* interaction with the host cell remain unclear. While there appear to be clear differences among isolates of

Cryptosporidium obtained from different sources, these differences at present are difficult to fully characterize or define phenotypically for the purpose of host specificity and speciation (Tzipori and Ward, 2002). The two major obstacles that hinder progress in this area are the inability to continuously propagate the parasite in vitro and the inability to cryopreserve the parasite, as the case with the majority of microorganisms.

Cryptosporidium parvum appears to make little effort to evade the immune system of the host. Many of the surface proteins, glycoproteins and phospholipids are strongly immunogenic, far more so than traditional enteric *Coccidia*, and many molecules on the surface of both sporozoites and merozoites are antigenically cross-reactive. The success of the parasite appears to be in its ability to develop rapidly and flood the environment with oocysts (Upton, 2003).

Cryptosporidium parvum exists in two distinct species; Genotype 1 (or genotype H for human) is termed *Cryptosporidium hominis* and is exclusively a parasite of humans (with a few minor exceptions) and Genotype 2 (genotype C for calf) is considered the traditional *Cryptosporidium parvum* and occurs in a wide variety of animals, including humans. The former species tends to be more aggressive in humans with a patent period (the length of time oocysts are shed in the faeces) nearly doubling that of genotype 2 and averaging just less than 2 weeks. Rarely, both species can be found infecting the same person or animal. Genetic markers on different chromosomes reveal there is little or no mixing between the two (i.e. isolates which are composed of mixed genotypes are not found), strongly supporting the notion that two distinct (but morphologically identical) species exist (Tyzzer, 2000).

2.4 Specificity of *Eimeria* species

Eimeria species demonstrate both site and host specificity; but to somewhat different degrees. The majority of species, for which endogenous development is known, undergo development within certain cells of the gastrointestinal tract, but not all species are found in this location. Once within their specific organ system of choice, *Eimeria* species seem

to be limited to specific zones within that system, specific cells within that zone, and specific locations within those cells. Thus one species may be found only in the middle third of the small intestine and another only in the cells of the cecum. Within their specific region, one species may be found only in the cells at the base of the crypts of Lieberkuhn, a second species in epithelial cell along the villi and a third species in endothelial cells of the lacteals in epithelial cell along the villi. Some species develop below the striated (microvillus) border of endothelial cells, but above the nucleus, others below the nucleus and a few within the nucleus (Duszynski and Wilber, 1997).

The degree of host specificity seems to vary between host groups; it has been studied best in mammals, and to a lesser degree in birds, especially domesticated stock/flock animals. *Eimeria species* from goats cannot be transmitted to sheep and vice versa (Dougald, 1979; Lindsay and Todd, 1993), but *Eimeria* from cattle (*Bos*) are found to infect American bison (*Bison*). Thus, numerous biotic interactions, particularly the genome of both parasite and host, must contribute to the host specificity, or lack thereof, attributed to each *Eimeria* species.

2.5 Etiology

Coccidiosis

Coccidiosis is contagious enteritis of predominantly young animals caused by infection with *Eimeria* species characterized by diarrhea and dysentery, anemia, inferior growth rates and production (Blood and Radostitis, 1989). It has been thought for many years that the *Coccidia* of sheep and goats are interchangeable. However, this is not the case for all species. Hence the species of clinical importance in sheep are *Eimeria ovina, Eimeria ahsata, Eimeria crandallis* and *Eimeria parva (*Long and Joyner, 1984*)*. In goats, species of *coccidia*, which are responsible for causing coccidiosis, are *Eimeria arloingi, Eimeria chrestenseni* and *Eimeria ninakohlyakimovae* (Soulsby, 1982). Moreover, multiple infections comprising more than a single species of *Coccidia* are the rule in natural infections (Parker and Jones, 1990). In faecal surveys in sheep and goats,

the prevalence of multiple species can be as high as 95% and 85%, respectively; where a single species of *Coccidia* may be the major pathogen but others probably contribute to the disease (Kennedy and Kralaka, 1987). The different species of *Eimeria* have oocysts varying in size and shape. For instance, *Eimeria ahsata* oocyst is ellipsoidal in shape and in average measures 33x24μm; *Eimeria faueri* is 29x21μm in size and it is ovoidal in shape (Jensen, 1988, and Richardson and Kendal, 1963).

Cryptosporidiosis

Cryptosporidiosis is caused by a minute, colorless and transparent protozoal parasite called *Cryptosporidium*.The genus *Cryptosporidium* is currently differentiated in to valid species based on their genetic profile and the species of the host from which they were originally isolated. Out of the thirteen valid species of *Cryptosporidium* that infect animals and man, only two species namely *Cryptosporidium parvum* and *Cryptosporidium andersoni* are involved in causing disease (Xiao *et al.*, 2004). *Cryptosporidium parvum*, measuring 5.0x4.5μm in size, infects the small intestine of an unusually wide range of mammals including humans, and is the zoonotic species responsible for approximately one half of human cryptosporidiosis (Tyzzer, 2000). *Cryptosporidium andersoni* infects the gastric glands of laboratory rodents and several other mammalian species. It is only rarely known to infect humans (Fayer, 1997). This species of *Cryptosporidium* is a bit bigger in size than *Cryptosporidium parvum* and measures 7.4x5.6μm (Bowman, 1995).

2.6 Life history

***Eimeria* species**

The life cycle includes both asexual and sexual multiplication. Sexual multiplication culminates in the formation of oocysts, which are discharged with faeces, and in the development, within each of these oocysts, four sporocysts each containing two infective organisms, sporozoites exist. If the infective sporulated oocyst is ingested by a suitable host, the sporozoites emerge and each may enter an epithelial or lamina propria cell, round up as a trophozoite, grow larger and become a first generation schizont (meront).

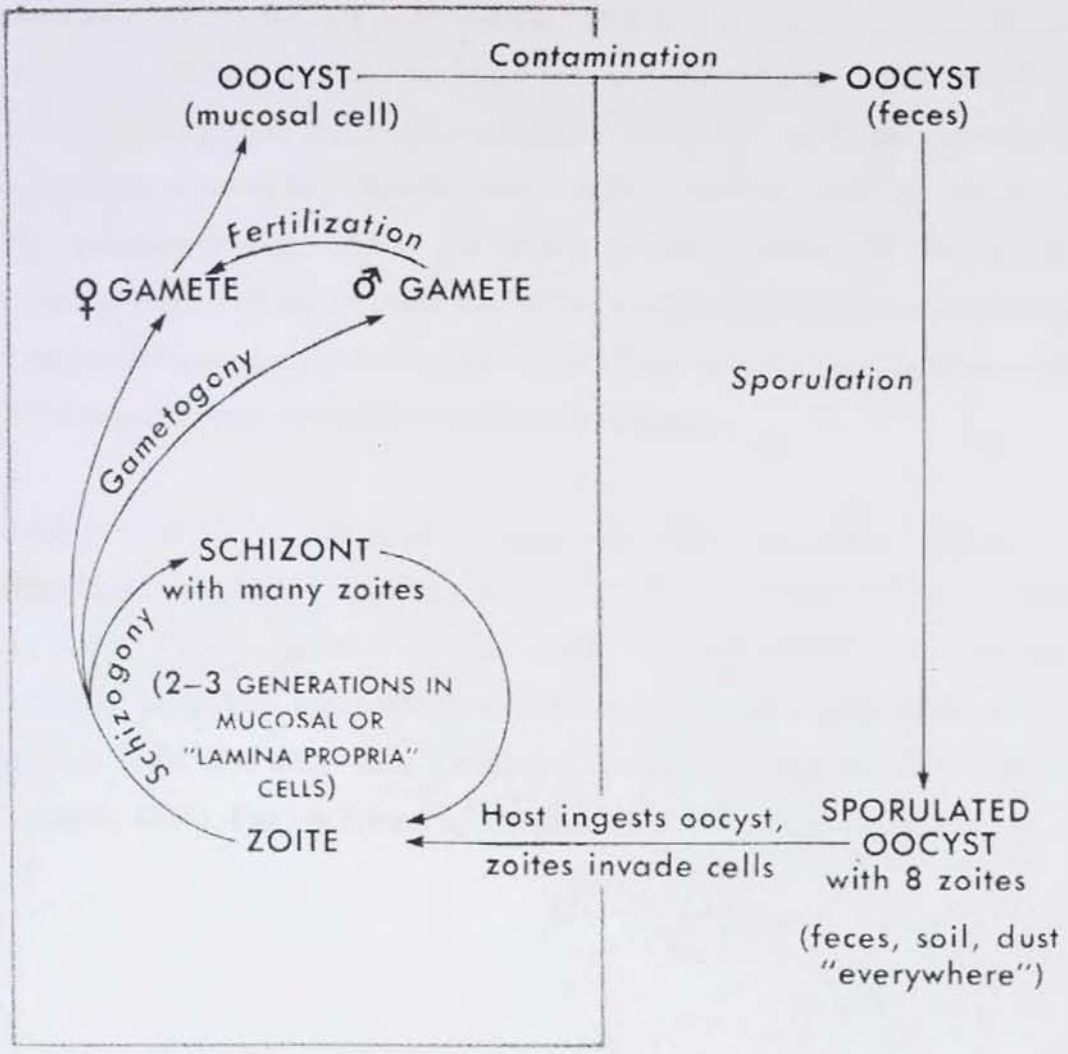
The schizont produces first generation merozoites that burst the cell and invade fresh cells to become second generation schizonts. A merozoite produced by the final schizogony enters a fresh host cell and develops in to either a male or a female gametocyte or developing sex cell.

The female gametocyte (macro gametocyte or macrogamete) enlarges, stores food materials and induces hypertrophy of both cytoplasm and nucleus of its host cell. When mature, it is called a macrogamete or female sex cell.

The male gametocyte (microgametocyte or microgamete) undergoes repeated nuclear division and becomes multinucleate. Each nucleus is finally incorporated in to a biflagellate microgamete or male sex cell. The microgametes fertilize macrogametes to form zygotes. A wall forms about the zygote by the coalescence of hyaline granules at its periphery to form an oocyst. The oocyst is released by rupture of the host cell and passes out with the faeces to undergo sporulation (Bowman, 1995). The life history of a typical *Eimeria* species is shown in figure 1 below.

Development of the *Eimeria* oocysts to the infective stage is dependent on suitable temperature, oxygen and moisture. In general, sporulation of oocysts is most rapid at 28°C to 31°C. Low temperatures of 0°C to 5° C retard sporulation, but it will then occur when the temperature is increased (Mary, 1990).

Figure 1 Life cycle of a typical *Eimeria* species (Bowman, 1995)

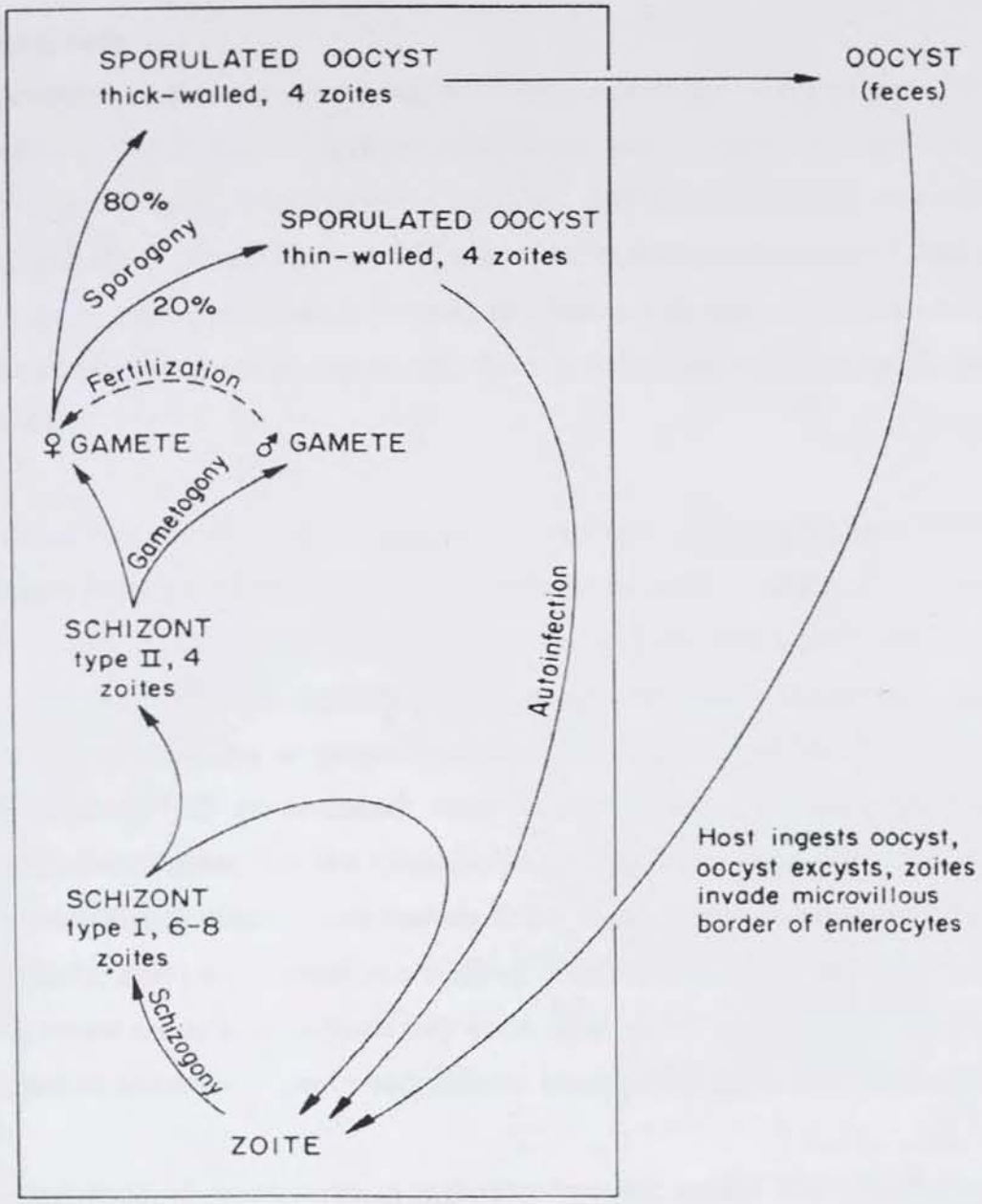


Cryptosporidium species

Infective, thick- walled oocysts containing four sporozoites are discharged in faeces and serve to disseminate the infection. The thick- walled oocysts remain viable for months unless exposed to extremes of temperature (below 0 °C, above 65 °C) and desiccation. Unlike Eimeria and Isospora species, which are intracellular, Cryptosporidium species are intramembraneous and resides within the brush boarder of the intestinal epithelial cells. When ingested by a suitable host, the thick walled oocyst excysts to release the four sporozoites that invade the micro villous boarder of the gastric glands (Cryptosporidium muris) or lower half of the small intestine (Cryptosporidium parvum). In the parasitophorous vacuoles of the micro villous boarder, the Cryptosporidians under go schizogony, gametogony, fertilization and sporogony.

About 20% of the oocysts produced have thin walls that break in the gastrointestinal lumen thus releasing their sporozoites, which in turn reinvade host cells. Therefore, the thin walled oocysts provide the mechanism for autoinfection that accounts for the chronicity of certain immunesufficient hosts and lethal hyper infection in immune deficient hosts. The other 80% are thick-walled oocysts that pass out with the faeces (Bowman, 1995). The life history of this parasite is indicated in figure 2 below.

Figure 2 Life cycle of cryptosporidium species (Bowman, 1995)



2.7 Epidemiology, source of infection and transmission

Coccidiosis

Coccidiosis occurs universally but is of most importance where animals are housed or confined in small areas. Shipping, ration change and severe weather are also considered to be predisposing factors to coccidiosis. All domestic animals are susceptible but the coccidia are in general host specific and infection does not pass readily from one animal species to another nor does cross immunity between species of *Coccidia* occur. Clinical disease is most common in cattle and sheep in which out breaks occur (Radostitis *et al.*, 1994).

Clinical coccidiosis of sheep and goats occurs mainly in young lambs and kids and there appears to be an increasing prevalence under conditions of intensive husbandry. Lambs are usually affected between four to seven weeks of age with a peak infection around six weeks. The out breaks reported have occurred where ewes and lambs were housed in unhygienic conditions or grazed intensively. Transmission is faecal-oral, either directly or indirectly via contaminated water or food washed or irrigated with faecally contaminated water. Un like *Cryptsporidia*, older animals usually serve as carriers of *Coccidia* and continue to pass oocysts in the faeces to the environment (Soulsby, 1982; Kennedy, 2001) which result in a build-up of infection in yards, barns and on pasture, so that severe and fatal coccidiosis may occur when a new batch of calves, lambs or kids is placed on pasture or in yard which hitherto has appeared perfectly safe (Soulsby, 1982).

The feeding of concentrates in stationary troughs, around which has occurred heavy contamination with oocysts can also be a precipitating factor. In the USA, coccidiosis has occurred when older lambs are confined in feedlots after weaning as a result of stress which is associated with shipping, sudden changes in ration and weather and over crowding (Radostitis *et al.*, 1994)). Coccidiosis can also occur in free-ranging conditions resulting from weather stress crowding around a limited water source as may occur during drought periods, which concentrates the hosts and the parasites within a restricted area (Aiello and Mays, 1998 and Ernst and Benz, 1986). In spring-lambing flocks in

Western Europe, infection of lambs results from oocysts, which have survived the winter and from those produced by ewes during the periparturient period (Urquhart *et al.*, 1996). Coccidiosis usually is sporadic during the wet seasons of the year, but may occur at any time in animals confined in feedlots. Sever losses have been reported in cattle confined in feedlots during periods of extremely cold weather (Aiello and Mays, 1998). The disease can be a problem at any time as long as conditions of adequate moisture exist for the survival and development of oocysts.

Goats appear to be very much susceptible and coccidiosis is a serious problem in raising kids in many goat herds. Clinical signs typically follow weaning by two or three weeks but coccidiosis should be suspected whenever diarrhea is observed in kids older than two weeks. Weaker, heavily infected kids are likely to die, the stronger less heavily infected survive but fail to grow normally (Bowman, 1995). Acute coccidiosis may occur in animals of any age when their resistance is affected by intercurrent disease or inclement weather (Blood and Radostitis, 1989).

Cryptosporidiosis

Cryptosporidiosis has been recognized worldwide primarily in neonatal calves but also in lambs, goat kids, foals and piglets. Older animals generally develop poor infections. This disease mainly affects animals between the first and third week of life. The infection originates even in the first twenty-four hours of life when the newborn animal ingests oocysts of the parasite (O'Donoghue, 1995). Although adult animals are considered as excreters of Cryptosporidium oocysts, their importance as sources of infection for the young remains questionable since oocyst excretion by adult animals was similar in herds with serious problems of Cryptosporidial neonatal diarrhea and in those with out (Anderson and Hall, 1982).

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; Mohamad *et al.*, 1999). In a Canadian study of beef calves, higher prevalence was found in winter and spring, the period related to calve season and consequently the

period with the greatest number of calves in the high-risk group (1-3 weeks old) (de Graaf *et al.*, 1996).

The oocysts *of Cryptosporidium* can sporulate within the host cell in contrast to oocysts of *Eimeria* and *Isospora* species which do not sporulate until they are passed out from the host and they are infective when passed in the faeces. The thick walled oocysts are resistant to most disinfectants including chlorine based compounds and can survive for several months in cool and moist conditions (Blood and Radostits, 1989). Very high concentration of disinfectants may be effective, but such levels are not practical for water treatment. They are quite common in rivers and lakes, especially where there has been sewage or animal contamination.

Since the parasite can cross host species barriers, infections in domestic animals, wild life species and companion animals must be regarded as possible reservoirs of infection (Blood and Radostits, 1989). *Cryptosporidium* oocysts are released in large quantities from clinically infected calves and humans (more than 10^{10} during acute or chronic infections), and less from other species of animals. Human and dairy effluents are probably the most important sources of environment and surface water contamination (Tzipori and Ward, 2002).

Cryptosporidiosis is transmitted by ingestion of oocysts excreted in the faeces of infected humans or animals. Hence infection can be transmitted from person to person, through ingestion of contaminated water (drinking water and water used for recreational purposes) or food, from animal to person, or by contact with faecaley contaminated environmental surfaces (Petersen, 1992).

2.8 Pathogenesis and clinical findings

Coccidiosis

The *Coccidia* of domestic animals pass through all stages of their life cycle in the alimentary mucosa and do not invade other organs although schizonts have been found in

mesenteric lymph nodes of sheep and goats (Blood and Radostitis, 1989). There have also been several reports of biliary coccidiosis with liver failure in dairy goats (Susan, 1998). *Eimeria* induces changes in the intestinal mucosa, the severity of which is related to the location of the parasites within the mucosa. The pathogenic lesions are mainly in ceacum and colon of heavily infected lambs. The lesions cause local haemorrhage and oedema: villus atrophy may be sequel resulting in malabsorption. Less is known about the problem of coccidiosis in goats even though oocysts are frequently recorded from the faeces and one species, *Eimeria arloingi*, has been reported as causing severe pathology (Urquhart *et al.*, 1996).

The clinical syndromes caused by various Coccidia are similar in all animal species .The first sign of clinical coccidiosis is usually the sudden onset of severe diarrhea with foul smelling, fluid faeces containing mucus and blood. In groups of lambs raised and fed under intensified conditions, the major clinical findings may be inferior growth rate, gradual onset of weakness, inappetance, recumbency, emaciation and death with a course of 1-3weeks (Blood and Radostitis, 1989). If a young kid is suddenly exposed to many sporulated oocysts it may become severely ill 1-2 weeks later. It will be off feed, listless, and weak. It may also show abdominal pain by crying or getting up again as soon as it lies down. Diarrhea begins pasty, then becomes watery. The animal may dehydrate rapidly and it may even be killed rapidly by a severe attack of coccidiosis (Smith *et al.*, 1992).

Cryptosporidiosis

Cryptosporidium is intercellular but extracytoplasmic. The pathogenesis of diarrhea is unknown but the varying degrees of villous atrophy suggests that the digestion and absorption of food may be impaired resulting in diarrhea. There is also evidence of hyperplastic crypt epithelium which along with damaged villous epithelium and atrophic villi indicates that the lesions develop as a result of accelerated destruction or loss rather than decreased production of epithelial cells (Blood and Radostits, 1989).

The faeces of diseased animals are usually foul smelling, yellow in color and the consistency ranging from pasty to liquid. These symptoms generally persist for 3-5 days in the midist cases and for 1-2 weeks in the more serious presentations (Riggs, 1997).
In addition to diarrhea, the affected animals also show a loss of appetite as a characteristic sign of the disease. The diminished milk intake is very marked at the start of the process and the animals can eventually reject feeding. This and the reduced utilization of nutrients by the damaged intestine cause the affected individual to lose weight (Troncoso, 1992).

2.9 Diagnosis

Coccidiosis

Diagnosis of coccidiosis is based upon the history (stress, high exposure), presence of clinical signs (untriftiness, diarrhea), the presence of large numbers of oocysts in the faeces and intestinal lesions at necropsy (Hall, 1977). For specific diagnosis of the parasite, one should depend on morphological identification of the oocysts in sugar flotation concentrates of faeces; micrometry; and sporulation of the oocysts in 2.5% potassium dichromate solution (Bowman, 1995). However, diagnosis will often be missed if one relies only on findings oocysts in the faeces. There may be none there at all in the acute stage of coccidiosis. Similarly, the mere presence of oocysts in the faeces is not proof that coccidiosis is present. To be sure of a diagnosis, scrapings should be made from the affected intestinal mucosa and examined under the microscope. It is not enough to look for oocysts; however, merozoites and gametes should be recognized (Levine, 1985). Some of the important morphological features of *Eimeria* species are presented in Annex 4.

In lambs which had previous contact with *Coccidia* and which may be relatively immune, other causes of diarrhea such as *E. coli*, *Salmonella species*, *Clostridium perfringens* type C and helmenthiasis should be considered (Blood and Radostits, 1989).

Cryptosporidiosis

Diagnosis of cryptosporidiosis is based on epidemiology of the disease, microscopic identification of sporulated oocysts in the faeces which could be accomplished by a simple semi quantitative microscopic slide floatation (MSF) method using modified sheather's sugar solution (Abassei *et al.*, 2000). Diagnosis can also be done by histological examination of intestinal tissue (gross pathologic lesions are not common) where it may reveal atrophy of the villi in the small intestine.

Cryptosporidium oocysts are difficult to see on faecal slides because they are colorless, transparent and very small in size. The oocysts walls have a pinkish hue that helps in finding them. Phase contrast microscopy is helpful and a number of staining procedures (Methylene blue, Giemsa stain, iodine wet mount and modified Kinyoun acid fast smear) have been recommended to increase the optical contrast and stain confusing yeasts differentially (Bowman, 1995).

A more accurate diagnosis is based on sophisticated staining techniques including immunofluorescence, antigen capture ELISA, Polymerase Chain Reaction (PCR) (Webster *et al.*, 1996); Gel electrophoresis and Western blotting (Lorenzo *et al.*, 1995).

2.10 Immunity

Coccidiosis

Specific immunity to each coccidian species develops after infection so that young animals exposed for the first time are often more susceptible to a severe infection and clinical disease than other animals (Radostits *et al.*, 1994). Even though resistance to reinfection with *Eimeria* species lasted for at least 3-6 months and possibly longer was demonstrated by different researchers, the immunity was not complete (Soulsby, 1982) and thus, frequent reinfection is required to boost up immunity (Blood and Radostits, 1989). The mechanism of the immune response is not fully understood, but it is thought to be a combination of cellular and humoral factors (Urquhart *et al.*, 1996). However, cellular immunity is probably more important in resistance against reinfection than

humoral immunity (Hughes, 1989, LAV, 1996). Since immunity to coccidiosis is species specific, animals recovered from a certain species of *Eimeria* are at risk of infection by other species (Rebhun, 1995) and the strength of immunity is different for the various species.

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 infection (O'Donoghue 1995; Abrahamsen, 1998). In contrast, severe chronic infections may develop in immunocompromised hosts with either congenital or acquired lymphocyte or gammaglobulin deficiencies there by suggesting that both cell-mediated and humoral responses are involved in the resolution of infections and 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).

2.11 Treatment, control and prevention

Coccidiosis

Prophylactic medication of feed and water supplies of feeder calves and lambs with most economical coccidiostats will control the disease and allow the development of immunity. Coccidiosis is a self limiting disease and clinical signs subside spontaneously when the multiplication stage of the parasite has passed. Sulpha drugs and amprolium are drugs that are mostly used for the treatment of clinical coccidiosis (Radostits et al., 1994). Recommended drugs for the treatment and control of coccidiosis are indicated in table 1below. Maintaining an adequate resistance level in the herd, controlling the parasite multiplication in the animal and reducing pasture contamination, can prevent coccidiosis. These could be achieved by the following control measures:

- Keeping susceptible animals away from contaminated pastures.
- Frequent rotation of pastures in groups of lambs at pasture.

- Avoiding the over crowding of animals.
- Lambing and calving grounds should be well drained and kept as dry as possible.
- Lambing pens should be kept dry-cleaned out frequently so that oocysts do not have time to sporulate and become infective.
- Routine effective immunity (Blood and Radostits, 1989).

Table 1 Recommended therapeutics for the treatment and control of coccidiosis in lambs and kids

Chemotherapeutic agent	Treatment	Prevention
Sulphadimidine	140 mg/kg body weight for three days individually	25 mg/kg bodyweight daily for one week
Nitrofurazone	15 mg/kg body weight daily for seven days or 0.04% in feed for 7 days. In water at 0.0133% for 7 days.	0.04% for 21 days in feed
Amprolium	Individual dose 50 mg/kg body weight for 4 days	50 mg/kg body weight in feed for 21 days
Monensin	2 mg/kg body weight daily for 20 days beginning on 13th day following experimental inoculation	20 mg/kg in feed fed continuosly
Lasalocid	-	25-100-mg/kg feed from weaning until market. Also in ewe's diet from 2 weeks before and Until 60 days after lambing.

Source: Blood and Radostits (1989)

Cryptosporidiosis

A large number of drugs have been used, including coccidiostatic agents and broad-spectrum antibiotics, though few have been shown to be effective. These drugs include spiramycin, halofuginone lactate, lasalocid and paramycin. The use of such products give rise to a partial reduction in the feacal elimination of oocysts and some times the associated diarrhea is less severe as a result (Haberkorn, 1996). Since the disease is self

limiting, supportive therapy such as rehydration and maintenance of energy requirements is usually sufficient (Heath, 1992).

Because all *Cryptosporidium* infections are initiated by the ingestion of environmentally resistant oocysts, control of this stage is the single most important factor in limiting the spread of the disease. Infected animals and humans will continue to contaminate the environment, and elimination of these sources is virtually impossible (Current and Gracia, 1991). Identification of the most common route(s) of transmission and a better understanding of the specific risk factor that leads to infection would greatly facilitate the development of a more targeted prevention strategy (Payment *et al.*, 1991).

Oocysts of *Cryptosporidium* are resistant to a variety of commercial disinfectants. Perhaps the single most effective and economical method of reducing the numbers of oocysts in the environment is simply by desiccation (Robertson *et al.*, 1992).

In humans, infection with *Cryptosporidium* species can be prevented by good sanitation and hygiene practices when handling young animals especially calves, lambs and kids. Infants, young children, or immunologically compromised individuals should not handle animals with diarrhea (Hendrix, 1998).

2.12 Public health significance of cryptosporidiosis

Cryptosporidiosis produces a transient, painful watery diarrhea in humans. Even though all ages are susceptible, the disease is pronounced in young children and the elderly. The duration of clinical signs in affected individuals varies considerably. Acute cases last from 3 to 7days, and chronic wasting syndromes can persist for weeks to a few months. Apart from diarrhea, clinical features of illness include nausea, low-grade fever, moderate abdominal cramps and anorexia (Reese *et al.*, 1982 and Payment *et al.*, 1991).

In industrialized nations, around 0.4% of the population appears to be passing oocysts in the faeces at any time. However, the seroprevalence is much higher and 30-35% of the

US populations have antibodies to *Cryptosporidium parvum*. In third world countries, the seroprevalence is even higher and up to 60-70% of people in these countries may have circulating antibodies to this pathogen. Since recent studies in the US have suggested serum antibodies to wane with time, it is likely that most adult humans have been infected with the parasite at least once in their lives. In AIDS patients, the number of individuals suffering from chronic cryptosporidiosis has been about 10% in industrialized nations and up to 40% in some third world countries (Tyzzer, 2000). The intestine is a large place for *Cryptosporidium* parasite and no one really knows how many oocysts it takes to establish an infection in humans. One study in the USA suggested that the 50% infectious dose in humans was around 132 oocysts (Tyzzer, 2000). Humans, like animals, appear to have various degrees of susceptibility to this parasite and the infective doses will probably be shown to vary between individuals and among isolates.

Most humans infected with *Cryptosporidium* species develop immunity and recover from the infection. In persons who are immunocomptent, the *Cryptosporidium* infection is limited to the small intestine. In immunocompromised individuals; however, the parasite can be found through out the gastrointestinal tract beginning from the esophagus to rectum, as well as in the hepatociliary system and respiratory tract (Sterling and Arrowood, 1992).

Infections with *Cryptosporidium* species may persist indefinitely in people with immunodeficiencies, particularly AIDS. The prognosis for immunologically compromised individuals with cryptosporidiosis is grave (Hendrix, 1998). Cryptosporidiosis can occur at any time in the course of HIV infection. However, sever and persistent disease correlates well with CD4 counts of less than 180 cells/mm^3 (Flanigan *et al.*, 1992).

2.13 The status of coccidiosis and cryptosporidiosis in the world

Small ruminant coccidiosis occurs world wide (Kaufmann, 1996; Radostitis *et al.*, 1994 and Urquhart *et al.*, 1996) but is of most importance where animals are housed or confined in small areas (Radostitis *et al.*, 1994). Despite its worldwide distribution, data on its prevalence are scant and are mostly reported from Europe and few ones are reported from Africa, and the prevalence ranges from 12.74 to 100 % (Sisodia *et al.*, 1997 and Zuzana *et al.*, 2004).

Cryptosporidiosis in small ruminants has also a worldwide distribution. It has been documented in people and animals in 95 countries (Fayer and Ungar, 1986). Most of the published reports were from Europe and little is known on the prevalence of small ruminant cryptosporidiosis in Africa. Some of the few reports are indicated in table 2.

Table 2 Prevalence of *Cryptosporidium* infection in sheep and goats in different countries

Serial No.	Country	Age involved	Animal Species	%	Source
1	Poland	≤ 1 year and > 1 year	Sheep	8.8	Majewska *et al.*, 2002
2	Poland	< 1 year	Sheep	24.32	Bogumila and Alexandra, 2002
3	Tanzania	> 1 year	Goats	0.8	Kambarage *et al.*, 1996
4	Poland	< 1 year	Sheep	10.1	Majewska *et al.*, 2000
5	Spain	1day to 3 months	Sheep (lambs)	59	Causape *et al.*, 2002
		> 1 year	Ewes	7.8	
6	Egypt	5 to 30 days	Sheep	13.3	Nassif *et al.*, 2002
			Goats	5.45	
7	Iraq	Not indicated	Sheep	13.3	Mahdi and Ali, 2002
			Goats	17.7	
8	Poland	< 1 year	Sheep	11.76	Pilarczyk and Balicka-Ramisz, 2001
9	Srilanka	< 6 months	Goats	28.5	Noordeen *et al.*, 2000
10	Srilanka	0-48 months	Goats	55	Noordeen *et al.*, 1999
11	Turkey	1-60 days	Sheep	2.97	Ferda *et al.*, 2005

2.14 The status of cryptosporidiosis and coccidiosis in Ethiopia

There are no reports of *Cryptosporidium* and *Eimeria* infections in small ruminants in this country except the few reports in other species of ruminant (cattle-calves). There are only two reports concerning the existence of *Cryptosporidium* in the country. One is that of Wudu (2004) who tried to determine the causes of calf morbidity and mortality through a longitudinal study. He reported a 6.7% prevalence of *Cryptosporidium* in diarrheaic calves with the age of 20-90 days in small and large-scale dairy farms located in Debre Zeit. The other report is that of Rahmeto (2005) who reported a prevalence of 17.6% in calves in selected dairy farms situated in Addis Ababa and Debre Zeit.

As far as *Coccidia* is concerned, there are only three reports from different parts of the country. These are a 20% prevalence report in calves over 2 months of age in small-scale dairy farms at Debre Zeit (Kebadu, 1998), a five-year retrospective laboratory report of 24.95% from Bahir Dar Regional Laboratory (Kassa *et al.*, 1987) and 68.1% prevalence of *Eimeria* infection in calves in dairy farms in Addis Ababa and Debre Zeit (Rahmeto, 2005).

3. MATERIALS AND METHODS

3.1. Description of the study area and study population

3.1.1. Study area

The study was conducted at ELFORA export abattoir, Debre-Zeit town and its surroundings. ELFORA export abattoir is a private firm located in Debre-zeit and is involved in slaughtering of cattle and small ruminants and exports the beef and mutton to the Middle East.

Debre Zeit is located at a distance of about 45km South East of the capital, Addis Ababa. The town is located at an altitude of 1850 meters above sea level and has a total human population of 95,000. It experiences a bimodal pattern of rain fall with a long rainy season from June to October and a short rainy season from March to May and has an average annual rainfall of 800mm. The area has an average maximum and minimum temperature of 27.7 ^{0}C and 12.3 ^{0}C, respectively (CACC, 2003). Debre-Zeit is the center of Ada'a Liben district, and according to the information obtained from the district agricultural and rural development office, the district has a total land area of about 161,056he and is divided in to three agro-ecological zones namely mid land (94%) high land (3%) and low land (3%).

3.1.2. Study population

The study population comprises of small ruminants that are intended for slaughter at ELFORA export abattoir and those which are raised under extensive condition in and around Debre- Zeit. Depending on the demand of the importing countries, more than 700 sheep, 800 goats and 65 cattle are brought especially from the Eastern and Southeastern regions of the country to the abattoir for slaughter per day. In Ada'a Liben district where Debre-zeit is the center, there are about 160,697 heads of cattle, 22,181 sheep, 37,510 goats, 1,660 horses, 38,726 donkeys, 268 mules, 191,380 poultry and 3,274 bee hives.

Out of the total sheep and goat population, about 2401 sheep and 1030 goats are urban holdings (CACC, 2003).

3.2. Study design

The study design utilized was a cross sectional epidemiological study.

3.3. Sampling methods and determination of sample size

The sampling method employed to select the sampling units for the ELFORA export abattoir was a systematic random sampling method where the sampling units were randomly selected at regular intervals from the population in such a way that every third animal was sampled.

The sample size was calculated based on the formula given by Thrusfield (1995) with a 95% confidence interval and at 5% desired absolute precision and is described as follows:

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

Where:

n = the required sample size

P_{exp} = expected prevalence

d = desired absolute precision

Since there was no previously done study to determine the prevalence of coccidiosis and cryptosporidiosis in sheep and goats in Ethiopia, the expected prevalence was taken as 50%. Thus, using the above the formula, the sample size for the ELFORA export abattoir was 384.

As for Debre -Zeit and its surrounding areas, the sampling method used was a stratified sampling technique considering the age of the study animals as strata. The age strata consisted of animals less than or equal to 1 year and greater than 1 year but less than or equal to 2 years old. The age of the animals were determined by looking in to their dentition pattern (Annex 1 and 2). The sample size used was the same as that of the systematic sampling method i. e. 384 animals. Since we considered two strata, the sample size for this study site was 384x2=768. In both the study areas a total of 1152 animals were sampled.

3.4 Study methodology

The study involved a combination of qualitative and quantitative faecal examinations.

3.4.1 Qualitative faecal examination

Faecal samples were taken directly from the rectum of the selected animals and examined microscopically by faecal flotation using concentrated sugar solution (Sheather's sugar solution) to detect the oocysts of *Eimeria* and *Cryptosporidium*. It also involved a microscopic examination of faecal smears suspected of *Cryptosporidium* using modified Kinyoun acid fast staining to differentiate it from yeasts (Kaufmann, 1996 and Hendrix, 1998).

Procedure for staining *Cryptosporidium* species in faecal smears (Kaufmann, 1996):

Thin faecal smears were air dried and passed quickly through a flame. The smears were stained with Ziehl- Neelsen's Carbol fuchsin solution for 2 minutes and then rinsed with tap water. The smears were rinsed for a few seconds with acid alcohol (3% hydrochloric acid in 70% ethanol). Again the smears were rinsed with tap water. The smears were counter stained with Brilliant Green (0.5%) for 2 minute. Rinse again with tap water. The slides were air dried and examined microscopically at 1000x using oil immersion. *Cryptosporidium* oocysts appeared bright red granules on a blue background.

3.4.2 Quantitative faecal examination

This procedure was performed to determine the number of oocysts found in a gram of faeces (OPG), which was important to assess the infection intensity of the parasites (Annex 9).

3.4.3 Faecal culture of coccidian oocysts for sporulation

When the unsporulated oocysts were recovered on faecal flotation, the observation was usually noted as *Coccidia*. Hence, sporulation of the oocysts in 2.5% potassium dichromate solution was conducted following the procedure described by Hendrix (1998) to identify *Eimeria* species from other similar oocysts like the Isospora(Annex 10).

3.5 Data analysis

The collected data was analyzed using SPSS (11.5 version) and Intercoded STATA (version 7) soft wares. The prevalence of infection was computed as the number of animals infected by either *Eimeria* or *Cryptosporidium* parasites divided by the number of animals sampled and multiplied by 100. The prevalence rate was computed at different levels such as study area, animal hosts, age, sex and season of the year.

The Pearson's chi-square (χ^2) was used as an screening test to see the association between the risk factors (variables) i.e. the study area, animal species, age, sex of the animal and season of the year and infection with either *Eimeria* or *Cryptosporidium* parasites, and a univariate and multivariate logistic regression analysis were followed to identify the major factors associated with the infections and to account for confounding factors. A statistically significant association between the variables and the infections was considered to exist if the calculated P-value is less than 0.05 at 95% confidence interval.

4. RESULTS

4.1 Assessment of the prevalence of Eimeria and Cryptosporidium infections

Faecal samples from a total of 1152 sheep and goats were collected and examined during the study period. Out of these, 346(49%) ovine and 173(38.5%) caprine were found to be infected with *Eimeria* species. Only faecal samples collected from four (0.57%) ovine were infected with *Cryptosporidium andersoni*. There was no *Cyptosporidium* species identified from caprine. The over all prevalence rates of *Eimeria* and *Cryptosporidium* infections were 45% and 0.35%, respectively (Table 3).

Table 3 The over all prevalence of *Eimeria* and *Cryptosporidium*

Animal host	No examined	No positive		(%)			
		Eimeria	*Cryptosporidium*	*Eimeria*	95% C.I	*Cryptosporidium*	95% C.I
Ovine	703	346	4	49	45-53	0.57	0.01-1.13
Caprine	449	173	0	38.5	34-43	0	0
Total	1152	519	4	45	42-48	0.35	0.01-0.69

4.2 Effect of study area on the prevalence rates of *Eimeria* and *Cryptosporidium* infections

Statistical analysis has indicated that there was a significant difference in the prevalence rate of *Eimeria* between the two study areas, the prevalence rate being higher (59.6%) at ELFORA export abattoir than Debre-zeit and its surroundings where the prevalence rate was 37.8% (Table 4). On the contrary, there was no statistically significant difference in

the prevalence rate of *Cryptosporidium andersoni* between the two sites. A negligible prevalence rate (0.5%) was recorded at Debre-zeit and its surroundings (Table 4).

Table 4 Prevalence rate of *Eimeria and Cryptosporidium* on study area basis

Study area	No. examined	(%)		C.I for percentage			
		Eimeria	*Cryptosporidium*	*Eimeria*		*Cryptosporidium*	
Debre Zeit and its surroundings	768	290(37.8)	4(0.5)	34	41	0.093	0.9073
ELFORA export abattoir	384	229(59.6)	0	54.7	64.5	0	
Total	1152	519(45)	4(0.35)	42	47.9	0.0089	0.69

χ^2=57.9436(P=0.000) P< 0.001 Significant for *Eimeria* species and χ^2= 2.0070 (P= 0.197) P> 0.05 not significant for *Cryptosporidium* species

4.3. Host difference in the prevalence rates of *Eimeria* and *Cryptosporidium* infections

There was statistically a significant difference (P<0.001) in the prevalence rate of *Eimeria* infection between ovine and caprine; with more infection rate (49%) in ovine and relatively less in caprine (38.5%)(Table 5). There was no significant difference in the prevalence rate of *Cryptosporidium andersoni* between sheep and goats (Table 6).

Table 5 Prevalence of *Eimeria* infection on the basis of animal host

Animal species	No. examined	No. positive	(%)	95% C.I	
Ovine	703	346	49	45	52
Caprine	449	173	38.5	34	43
Total	1152	519	45	42	47.9

χ^2=14.4518 (P=0.000) P< 0.001 Significant

Table 6 Prevalence of *Cryptosporidium* species on the basis of animal host

Animal species	No examined	No positive	(%)
Ovine	703	4	0.57
Caprine	449	0	0
Total	1152	4	0.35

χ^2=2.5919 (P=0.138) P> 0.05 Not significant

4.4. The interaction between age of study animals and prevalence rates of Eimeria and Cryptosporidium infections

In this study, it was observed that young animals with the age of less than or equal to 12months were found to be more infected with *Eimeria* species (57%) than the adult ones (19.5%) (Table 7). However, there was no statistically significant difference in the prevalence rate of *Cryptosporidium andersoni* between the two age categories indicating a relatively equal chance of exposure to *Cryptosporidium* infection (Table 7).

Table 7 Prevalence of *Eimeria and Cryptosporidium by* age

Age category (Month)	No. examined	(%)	
		Eimeria species	*Cryptosporidium* species
≤ 12	783	447(57)	3(0.39)
> 12 -≤ 24	369	72(19.5)	1(0.27)
Total	1152	519(45)	4(0.35)

χ^2 =150.8008 (P= 0.000) P< 0.001 Significant for *Eimeria* species and χ^2 =0.0938 (P=0.612) P> 0.05 not significant for *Cryptosporidium*

4.5. Seasonal variation

Since animals intended for slaughter at ELFORA export abattoir were originated from areas with extremely different climatic conditions (Temperature, humidity etc.), statistical analysis for the differences in the prevalence rates of *Eimeria* and *Cryptosporidium* among the five months of study period was not computed. As a result, seasonal variation in infection rate was analyzed only for samples collected from Debre-zeit and its surroundings. Accordingly, statistical analysis has indicated a significant variation in the prevalence rate of *Eimeria* species during the study period with the highest infection rate (69.8%) in October and the lowest in February (22%) indicating that the prevalence rate followed a descending order of pattern from October to February (Figure 3).

Nevertheless, there was no statistically significant difference observed in the prevalence rate of *Cryptosporidium andersoni* among the months of the study period. *Cryptosporidium andersoni* was not identified during the study period except in January when a prevalence rate of 1.3% was registered (Table 8).

Figure 3 Monthly Prevalence of *Eimeria* infection at Debre-zeit and its surroundings

Table 8 Monthly Prevalence of *Cryptosporidium* infection

Month	No examined	No positive	(%)
October	43	0	0
November	103	0	0
December	140	0	0
January	310	4	1.3
February	172	0	0
Total	768	4	0.5

χ^2=7.8426 (P=0.204) P> 0.05 Not significant

4.6.Sex prevalence interaction

Statistical analysis revealed that there was no significant difference (P> 0.05) in the prevalence rate of both *Eimeria* and *Cryptosporidium* between the two sexes (Table 9). Since only male sheep and goats were brought to ELFORA export abattoir for slaughter, the sex prevalence interaction has not been included for ELFORA export abattoir.

Table 9 Prevalence of *Eimeria* and *Cryptosporidium species* at Debre-zeit and its surroundings by sex

Sex	No. examined	%	
		Eimeria species	*Cryptosporidium species*
Female	373	144(38.6%)	2(0.53%)
Male	395	146(36.9%)	2(0.5%)
Total	768	290((37.8%)	4(0.52%)

χ^2=0.0022 (P=0.346) P> 0.05 for *Eimeria* species and χ^2=0.3417 (P=0.666) P> 0.05 for *Cryptosporidium* species

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

Factors	Odds ratio	Std.Err	Z	P>|Z|	[95%conf. Interval]	
Eimeria species						
Study site	1.859207	0.2577827	4.47	0.000**	1.416794	2.439768
Season (Months)	0.7148455	0.0414047	-5.80	0.000**	0.6381306	0.800783
Animal host	0.6817283	0.0920772	-2.84	0.005**	0.5231718	0.8883381
Age	0.2043649	0.031862	-10.18	0.000**	0.1505558	0.2774055
Cryptosporidium						
Season	0.0552204	0.8359483	0.82	0.414	0.04401684	0.05660343
Age	0.05873789	0.6909503	-0.45	0.651	0.0585634	0.0891292

** **The association is significant at 5% level**

4.7. Results of parasite species identification

Twelve and ten different *Eimeria* species were identified respectively in sheep and goats based on the morphological characteristics of oocysts (Size, shape, presence or absence of micropyle and the polar cap) (Tables 11 and 12). *Eimeria parva* with the prevalence rate of 30.8%, *Eimeria crandallis* (30%) and *Eimeria pallida* (13.8%) were the three most prevailing species in sheep while *Eimeria arloingi* (31%), *Eimeria ninakohlyakimovae* (18.5%) and *Eimeria faurei* (10.8%) were the most frequently observed species in goats. *Eimeria parva*, *Eimeria pallida*, *Eimeria hawkinsi* and *Eimeria faurei* were found to infect both the animal species.

Table 11 *Eimeria* species identified from sheep and their respective prevalence in descending order

Eimeria species	Frequency of occurrence	(%)
Eimeria parva	132	30.8
Eimeria crandallis	130	30
Eimeria pallida	59	13.8
Eimeria weibridgensis	31	7.2
Eimeria ovina	22	5
Eimeria intricata	11	2.6
Eimeria punctata	9	2.1
Eimeria faurei	9	2.1
Eimeria marsica	8	1.9
Eimeria ahsata	7	1.6
Eimeria granulosa	5	1.2
Eimera hawkinsi	2	0.5

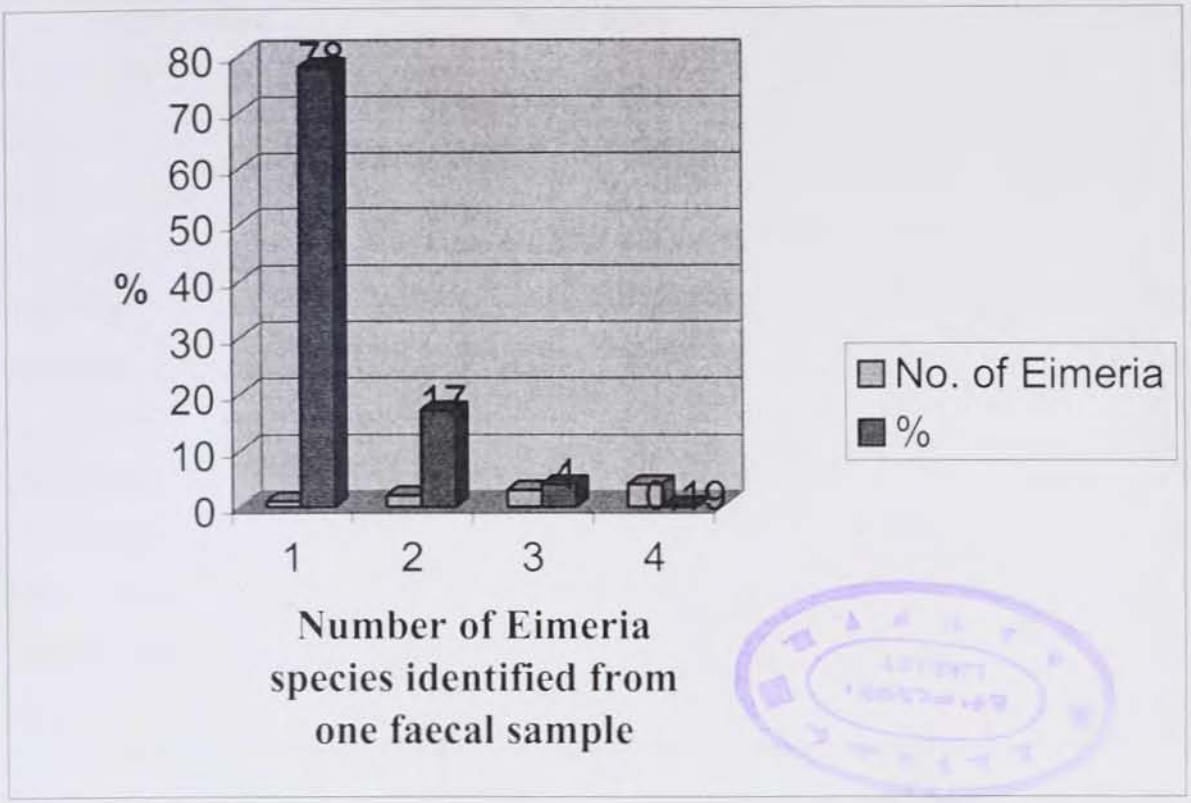
Table 12 *Eimeria* species identified from goats in descending order of prevalence

***Eimeria* species**	**Frequency of occurrence**	**(%)**
Eimeria arloingi	69	31
Eimeria ninakohlyakimovae	41	18.5
Eimeria faurei	24	10.8
Eimeria christenseni	22	9.9
Eimeria parva	18	8
Eimeria hirci	15	6.8
Eimeria palida	14	6.3
Eimeria caprina	13	5.8
Eimeria alijevi	4	1.8
Eimeria hawkinsi	2	0.9

Though the majority of the animals 408 (78%) were infected with only one type of *Eimeria* species, mixed infections with more than one *Eimeria* species in both animals were also observed. Eighty-nine animals (17%), 21 animals (4%) and one animal (0.19%) were infected with 2, 3 and 4 *Eimeria* species respectively (Figure 4).

Cryptosporidium was identified only from sheep raised under extensive grazing system of Debre Zeit and its surroundings. The species identified was *Cryptosporidium andersoni* formerly known as *Cryptosporidium muris*. The most pathogenic and the species mostly responsible for human cryptosporidiosis, *Cryptosporidium parvum*, was not identified by the current study. The observed prevalence of *Cryptosporidium* was very low (0.35%).

Figure 4 The frequency of occurrence of *Eimeria* species per faecal sample



4.8.Quantitative faecal examination

The oocyst count per gram of faeces (OPG) was conducted using the Mac Master technique, which revealed a minimum and maximum OPG values of 0 and 2000,0000 respectively with the mean of 3955.642. Majority of the samples (58.5%) had an OPG value ranging from 0 to 100, and only few samples (0.2%) had OPG value above 200,000 implying a low infection intensity of this parasite. The summary result of the OPG count is indicated on Table 13.

Table 13 Result of oocyst count per gram of faeces (OPG)

OPG value	No of cases	(%)
0-100	674	58.5
1001-1000	252	21.9
1001-2000	119	10.3
2001-3000	26	2.3
3001-4000	18	1.6
4001-5000	9	0.8
5001-10000	19	1.6
10001-20000	23	2
20001-30000	5	0.4
30001-40000	3	0.3
101000-200000	2	0.2
>200000	2	0.2

Statistically there was no significant difference in the mean OPG values of *Eimeria* between the two age categories (t=1.3663, P> 0.05) (Table 14). There was a significant difference in the mean OPG values of *Eimeria* among the months of the study period. The highest and lowest mean OPG values were observed in the months of November and December respectively.

Table 14 T-test analysis of the association between age and OPG of *Eimeria* species

Age (Month)	Mean	Std.Error Of mean	Std.Dev	[95% Conf. Interval]		t	df	P
≤ 12	5781.226	2869.805	80303.27	147.7932	11414.66	1.3663	1150	0.1721
> 12-≤ 24	68.02168	11.33363	217.712	45.73487	90.30849			
Total	3951.215	1951.757	66244.82	121.8157	7780.615			

5. DISSCUSSION

5.1 *Eimeria* species

In this study, out of the 703 sheep and 449 goats examined, 346 (49%) sheep and 173 (38.5%) goats were found to be infected with different *Eimeria* species. This finding is the first documented report regarding the prevalence of *Eimeria* infection in small ruminants of Ethiopia.

Various prevalence rates of *Eimeria* infection in sheep and goats have been reported in various parts of the world. Kambarage *et al.* (1996) reported a 97.5% prevalence in sheep, 97.3% in goats in Tanzania; Kusiluka *et al.* (1996) recorded 91% and 93% prevalence rates in sheep and goats respectively in Tanzania; Arslan *et al.* (1999) obtained a prevalence rate of 93.9% in sheep of Turkey; Serdar *et al.* (2003) reported a prevalence rate of 73.6% in goats in Turkey; Galip (2004) recorded 100% prevalence in sheep in Turkey; Balicka-Ramsiz (1996) reported 90.5% in goats in Poland; Mahmoud *et al.* (2003) recorded 54% prevalence in goats in Jordan; Shivario (2003) reported a 50% prevalence of *Eimeria* infection in goats in Kenya while Waruru *et al.* (2004) recorded 28% prevalence in goats in the same country; Hathuy Hanh and Vu Dang Dong (2003) obtained 36.84% prevalence in goats in Turkey; Kaarma and Maji (2002) reported 50% in sheep in Estonia; Ola-Davies *et al.* (2002) recorded 59.6% in goats in Nigeria; Ashokkumar *et al.*(2001) reported 58.43% in goats in India; Hassum and Menezes (1999) reported 81.95% in goats in Brazil; Divanoic *et al.*(1999) recorded 16.6% prevalence in goats in Croatia; Sharma *et al.* (1997) reported 25% in goats in India; Woji *et al.* (1994) recorded a prevalence of 87% in goats in Nigeria; Sisodia *et al.* (1997) reported 12.7% prevalence in sheep in India and Harper and Penzhorn (1999) reported 88.7% prevalence of *Eimeria* infection in goats in South Africa.Vercruysse (1982) in Senegal reported 94% and 85% prevalence of *Eimeria* infection in sheep and goats respectively. On the other hand, Zuzana *et al.* (2004) recorded a 100% prevalence of this infection in sheep and goats in Slovakia.

The present finding in the prevalence of *Eimeria* species infection in sheep (49%) is lower as compared to that reported by Kambarage *et al.* (97.5%), Kusiluka *et al.* (93%), Arslan *et al.* (93.9%), Galipkaya (100%) but greater than that reported by Sisodia *et al.* (12.74%). The current finding is relatively similar to the findings of Kaarma and Maji (50%) and Mahmoud *et al.* (54%). As far as goats are concerned, the present finding i.e. 38.5% is lower as compared to that reported by Kambarage *et al.* (97.3%), Kusiluka *et al.* (91%), Serdar *et al.* (73.6%), Balicka-Ramisz (90.5%), Hassum and Menezes (81.95%), Woji *et al.* (87%) and Harper and Penzhorn (88.7%) but greater than that of Sharma *et al.* (25%) and Divanovic *et al.* (16.62%). The present finding is relatively similar to the findings of Ha Thuy Hanh and Vu Dang Dong (36.84%), Ola-Davis *et al.* (59.6%), Ashokkumar *et al.* (58.43%) and Shivario (50%).

Statistical analysis has indicated that there was a significant variation in the prevalence of *Eimeria* infections between the two study areas ($P< 0.001$); with more prevalence rate (59.6%) at ELFORA export abattoir and relatively less (37.8%) in animals sampled from Debre-zeit and its surroundings. The high prevalence rate at ELFORA export abattoir could be attributed to transport and crowding stresses during transportation from their origin to the abattoir. Animals intended for slaughter are usually collected from different areas of the country and are kept at different holding centers till they are transported to abattoirs and hence they may also be exposed to high doses of sporulated oocysts that were passed out with the faeces from the previous batches of animals in the holding centers.

There was a significant difference ($P< 0.001$) in the prevalence of *Eimeria* infection between the two species of the study animals (Table 5); the prevalence rate being higher in sheep (49%) than goats (38.5%). This disparity could emanate from the differences in the natural immunity of the hosts and from the feeding habits of these animals. Goats are usually browsers in nature and they tend to graze in very rare cases where they do not find shrubs and bushes; thereby reducing the risk of being infected with sporulated oocyts of *Eimeria* species and other internal parasites. A similar finding was obtained by

Waruru *et al.* (2004) in which higher prevalence of *Eimeria* infection was observed in sheep than goats.

Young animals were found to be more exposed to *Eimeria* infection than adults ($P< 0.001$). A prevalence rate of 57% was recorded in animals aged less than or equal to 12 months while animals above 12 months to less than or equal to 24 months age had 19.5% prevalence rate (Table 7). Similar results were also reported by Waruru *et al.* (2004), Mahmoud *et al.* (66% in young goats and 49% in adult ones) and by Balicka-Ramisz (100% in young and 81% in adult goats). This is perhaps associated with the immunity of the animals where adult animals have a better immunity due to previous exposure to *Eimeria* infection compared to young animals, which are usually susceptible to initial infections (Radostitis *et al.*, 1994).

A significant variation in the prevalence of *Eimeria* infection was observed during the study period in Debre Zeit and its surroundings ($P< 0.001$). The highest prevalence rate was observed in October (69.8%) and has steadily declined in the following months. In October, the prevailing climatic conditions (Humidity and temperature) are usually conducive for oocyst sporulation and thus posing high risk of *Eimeria* infection to animals. Since the life cycle of *Eimeria* is relatively short in duration, the favourable climatic condition in the period earlier to October might also contribute to the high prevalence of *Eimeria* infection. In the months of January and February, when the weather was dry, infection rates were relatively low (30% and 22% respectively). In dry weather condition oocysts normally desicate by sunlight and sporulation will be affected. There was no significant difference ($P> 0.05$) observed between the two sexes of animals in the prevalence of *Eimeria* infection indicating both sexes to be equally susceptible.

In the present study, a total of 12 *Eimeria* species in sheep and 10 species in goats have been identified based on the characteristics of the oocysts as described by Soulsby (1982); Levine (1985). This suggests the abundance of *Eimeria* species in the study areas.

Mixed infections in a single host were common findings in this study suggesting the prevailing condition of polyparasitism. The maximum number of *Eimeria* species per sample was four as in indicated in figure 4. Several works have been conducted to identify *Eimeria* species by several researchers overseas. Galip, (2004) identified 10 different *Eimeria* species from lambs in Antakya province of Turkey where *Eimeria crandallis* (64.91%), *Eimeria ovinoidalis* (55.24%) and *Eimeria bakuensis* (38.7%) were the most prevailing species. Gul and Deger (2002) reported nine different species in sheep in Turkey; *Eimeria parva* (46.7%), *Eimeria ovinoidalis* (43.14%), *Eimeria ahsata* (39.42%) and *Eimeria ovina* (39.14%) being the most prevalent species.

Sisodia *et al.* (1997) has also reported 7 different *Eimeria* species namely *Eimeria ovina* (24%), *Eimeria ovinoidalis* (22%), *Eimeria crandallis* (16%), *Eimeria parva* (14%), *Emeria intericata* (12%), *Eimeria ahsata* (0.6%) and *Eimeria pallida* (0.6%) from sheep examined in India. On the other hand, Kusiluka *et al.* (1996) identified 7 *Eimeria* species from sheep in Tanzania; *Eimeria crandallis* (96%), *Eimeria parva* (92%) and *Eimeria ovinoidalis* (29%) being the most frequently observed species.

In this study, *Eimeria parva* (30.8%) was found to be the most frequently encountered species in sheep, which is consistent with the finding of Gul and Deger (2002). *Eimeria ovinoidalis*, *Eimeria gonzalezi* and *Eimeria gilruthi* were not identified in this study. *Eimeria marsica* and *Eimeria hawkinsi*, which were not identified by the above mentioned workers, have been identified in this study. *Eimeria crandallis*, the second most prevalent species in this study was also the most frequently observed species in the study of Galip, (2004) and Kusiluka *et al.* (1996) indicating the worldwide distribution of these species.

Among the 10 different *Eimeria* species identified in goats, the most pathogenic species, *Eimeria arloingi* (31%) and *Eimeria ninakohlyakimovae* (18.5%) were the most frequently encountered species. This shows that there will be a considerable threat of goat coccidiosis in this country if intensification programmes in the livestock sector particularly in the areas of small ruminants are implemented.

Several species of *Eimeria* were reported to exist in goats. Waruru *et al.* (2004) reported 7 species in goats in Kenya; where *Eimeria ninakohlyakimovae* (45.9%) and *Eimeria arloingi* (26.1%) were found to be the most prevailing species. Serdar *et al.* (2003) identified nine different *Eimeria* species in goats in Turkey: *Eimeria arloingi* (40.9%), *Eimeria christenseni* (34.4%), *Eimeria alijevi* (32.6%), *Eimeria pallida* (31%), *Eimeria hirci* (30.2%), *Eimeria ninakohylyakimovae* (29.8%), *Eimeria jolchijevi* (26%), *Eimeria apsheronica* (5.8%) and *Eimeria punctata* (0.8%).

Mahmoud *et al.* (2003) from Jordan identified 8 species of *Eimeria* from goats in which *Eimeria ninakohlyakimovae* (19%), *Eimeria caprina* (15%) and *Eimeria apsheronica* (15%) were the most prevalent species. Ten *Eimeria* species; *Eimeria arloingi* (98.7%) and *Eimeria hirci* (79.7%) with high occurrence were recovered from faecal samples of goats in South Africa (Harper an Penzhorn, 1999). *Emeria christenseni, Eimeria arloingi, Eimeria jolchijevi* and *Eimeria ninakohlyakimovae* were found to be mostly occurring in Poland (Balicka-Ramsiz, 1999). Agyeei *et al.* (2004) further reported *Eimeria arloingi* (20.5%) and *Eimeria ninakohlyakimovae* (17.02%) to be dominant species in West African dwarf kids in Ghana.

The frequent finding of *Eimeria arloingi* and *Eimeria ninkohlyakimovae* in this study was consistent with the reports of most authors described earlier. In this study, *Eimeria caprovina, Eimeria apsheronica* and *Eimeria jolchijevi* were not identified. In both sheep and goats, clinical coccidiosis was not observed in this study.

5.2. *Cryptosporidium* species

Out of the 1152 faecal samples collected during this study, only four animals (0.35%) were found to be infected with *Cryptosporidium*. This is the first documented report regarding the presence of *Cryptosporidium* in sheep of this country. Several reports are available on the prevalence of this infection overseas; most of which are from Europe and few reports from Africa. Freda *et al.* (2005) reported 2.97% prevalence rate of *Cryptosporidium parvum* in 1- 60-day-old lambs in Turkey; Majewska *et al.* (1998) recorded 8.8% prevalence in sheep in Poland; 0.8% prevalence was reported in goats from Tanzania (Kambarage *et al.*, 1996). Bogumila and Alexandra (2002) reported 24.32 % of *Cryptosporidium* oocysts in lambs of Poland; Majaweka *et al.* (2000) again reported 10.1% prevalence from sheep in Poland; Causape *et al.* (2002) reported 59% and 7.8% prevalence rates in lambs and ewes respectively from Spain.

In addition, Nasiif *et al.* (2002) recorded 13.3% and 5.45% prevalence rates in sheep and goats respectively from Egypt. Mahdi and Ali (2002) obtained prevalence rates of *Cryptosporidium* infection of 13.3% and 17.7% in sheep and goats respectively in Basrah, Iraq; Pilarczyk and Balicka-Ramisz (2001) recorded 11.76% prevalence in lambs in Poland. Further more, Noordeen *et al.* (1999) reported 55% prevalence in 1999 and 28.5% in the year 2000 in goats of different age groups in Srilanka.

The present finding in the prevalence rate of *Cryptosporidium* infection (0.35%) is very low compared to the results of different authors described above except that reported by Kambarage *et al.* (1996) (0.8%) from Tanzania, which is comparatively similar to the present result.

The lower prevalence of *Cryptosporidium* infection in this study is perhaps due to the intermittent excretion of *Cryptosporidium* oocysts (Casemore *et al.*, 1997), the difficulty in detecting oocysts in faeces,the type of study design (Cross sectional type of study design), which only gives a momentary picture of prevalence rate (Thrusfield, 1995).

Since 373 adult animals (Greater than one year old) were also included in this study, the prevalence rate of *Cryptosporidium* infection might have been underestimated, as adult animals are usually immune to *Cryptosporidium* infection as a result of previous exposure during their lifetime.

It was only from animals sampled from Debre Zeit and its surrounding areas that *Cryptosporidium* ooccysts were diagnosed. There was no *Cryptosporidium* identified from faecal samples collected from small ruminants intended for slaughter at ELFORA export abattoir. *Cryptosporidium* oocysts were not identified from goats during this study period.

Statistically, there was no significant difference ($P > 0.05$) observed in the prevalence rate of *Cryptosporidium* between the study areas, animal species (sheep and goats) between different age groups (Adult and young), between animal sexes, among the months of study period. *Cryptosporidium parvum*, the most pathogenic and zoonotic species was not identified in this study.

6. CONCLUSION AND RECOMENDATIONS

This study has revealed that *Eimeria* infection is common in sheep and goats of Debre-zeit and its surroundings, and in those intended for slaughter at ELFORA export abattoir. Based on the morphology of *Eimeria* oocysts, a total of twelve and ten different *Eimeria* species in sheep and goats were respectively identified; of which *Eimeria parva*, *Eimeria crandallis* and *Eimeria pallida* were the dominant species in sheep; while *Eimeria arloingi*, *Eimeria ninakohlyakimovae* and *Eimeria faurei* were found to be the most frequently encountered species in goats.

A low prevalence rate of *Cryptosporidium* infection was registered in this study from faecal samples collected from sheep in Debre-zeit and its surroundings. *Cryptosporidium* was not identified from animals sampled from ELFORA export abattoir. In addition, *Cryptosporidium* was not identified from faecal samples collected from goats during this study. Even though most of the animals examined were found to be infected with Eimeria species, clinical coccidiosis was not observed in any of the infected animals. This shows that most *Eimeria* infections result in sub clinical form of coccidiosis which can still negatively influence the productivity of animals and cause economic loses as a result of poor growth, slow weight gain, weight loss, low feed efficiency and increased susceptibility to other infectious diseases.

In this study, four major factors were found to be associated with the risk of infection with *Eimeria*. These include the study area, season of the study period, animal host and the age of the animals sampled. Higher prevalence rate of *Eimeria* infection was observed in animals sampled from ELFORA export abattoir than from those sampled from Debre-zeit and its surroundings. High *Eimeria* infection rate in October was recorded and the prevalence rate steadily declined in the following months of the study period. More prevalence rate of *Eimeria* infection was registered in sheep than in goats implying that sheep were frequently infected with *Eimeria* oocysts. Younger animals were found to be frequently infected with *Eimeria* than the adult ones.

In this study, high mean OPG value of *Eimeria* was registered in the month of November and the least figure was recorded in December. There was no significant difference observed in the mean OPG values of *Eimeria* between the two age categories compared, suggesting the relative absence of difference in the infection intensity of this parasite between young and adult animals. In the final analysis, *Cryptosporidium* infection was found to be not associated with any of the factors studied during this study. Based on the present findings, the following recommendations are forwarded:

- Even though low prevalence rate of *Cryptosporidium* infection was recorded in this study, creation of awareness on the possible existence of this zoonotic parasite is essential.
- During planning and implementation of the establishment of small ruminant ranches or farms in this country, prediction of the possible occurrence of coccidiosis is important in order to device appropriate control and preventive strategies as those pathogenic *Eimeria* species have been identified by this study.
- A better diagnostic approach (For example, molecular technique) is required to precisely confirm the species of *Cryptosporidium* identified, as the present identification was based on conventional laboratory procedures.
- Further study based on different diagnostic approaches (Morphological characteristics, sporulation time determination and necropsy examination of the gut) is required for more precise identification of the species of *Eimeria* circulating in small ruminants of Ethiopia.
- As this work was a cross sectional type of epidemiological study, further longitudinal study with repeated samplings is required to obtain a more accurate data on the prevalence and economic impacts of these parasites.

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8. ANNEXES

Annex 1 Estimation of age of Sheep (Gatenby, 1991)

Permanent incisors	Age
None	Less than 1 year and 3 months
1 pair	1 year and 3 months up to less than 1 year and 10 months
2 pairs	1 year and 10 months up to less than 2 years and 4 months
3 pairs	2 years and 4 months up to 3 years
4 pairs	More than 3 years

Annex 2 Determination of the age of goats (Mike, 1996)

Age group	Teeth condition
Kids under 1 year	8 sharp incisors
Yearlings (1-2 years)	Centeral pair of baby teeth replaced by permanent ones
Young adults (3-4years)	4 permanent teeth
Adult (4-5 years)	8 permanent teeth
Older adults more than 5 years	Worn teeth and some missing

Annex 3 List of laboratory reagents

3.1. Reagents for sheathger's sugar solution preparation

Granulated sugar (Sucrose): 454gm

Tap water: 355ml

Formaldehyde (40%): 6ml

3.2. Reagents for Kinyoun acid-fast stain

For the preparation of Carbol fuschine

Basic fuchsine: 4gm

Phenol (Melted crystal): 8ml

Ethanol (95%): 20ml

Distilled water: 100ml

For the preparation of decolorizer

Ethanol (95%): 97ml

HCL (concentrated): 3ml

For the preparation of counterstain

Methylene blue: 0.3gm

Distilled water: 100ml

Annex 4 *Coccidia* of sheep and goats (Soulsby, 1982 and Levine, 1985)

Serial No.	*Eimeria spp.*	Host	Size of oocysts in micrometer	Shape of oocysts	Other characteristics
1	*E. ahsata*	Sheep	32.7x23.7 in forms from the big horn sheep; 33.4x22.6 in forms from domestic	Ellipsoidal	• Oocysts wall smoth, pinkish yellow, a dome-shaped polar cap over the

			sheep		micropyle • Prepatent period of 18-20 days
2	*E. arkhari*	Wild sheep	22.4x17.4	Ellipsoidal to oval	Double contoured oocyst wall with a yellow tint, micropyle absent
3	*E. arloingi*	Goats	27x18 but a wide range occurs: 17-42x13-31; 22-31x17-22 with a mean of 28x20	Ellipsoidal	- Distinct polar cap - Sporulation time of 48-72 hours
4	*E. christenseni*	Goats	38x25; range of 34-41x23-28	Ovoid and slightly flat at one end	Micropyle covered by a prominent dome-shaped micropyle cap
5	*E. cranadallis*	Sheep	23x19 range 20-27x17-20	Spherical to broadly ellipsoidal	Visible micropylar cap
6	*E. danielle*	Sheep	-	-	-
7	*E. faurei*	Sheep and goats	28.9x21 range 25-33x18-24	Ovoidal	- Distinct micropyle - No polar cap - Wall transparent, brownish

					yellow to salmon pink
8	*E. gilruthi*	Sheep and goats	-	-	-
9	*E. gonzalezi*	Sheep	26-38x20-26(mean 30.5x22.4)	Ellipsoidal or ovoid	- Wall of oocyst smooth with transparent yellow outer layer - Prominent micropyle covered by a micropylar cap
10	*E. granulosa*	Sheep	Mean size of 29.4x20.9 range 22-35x17-25	Urn-shaped	- Distinct micropyle with a micropylar cap - Oocyst wall transparent, brownish to yellowish

					in color - Sporulation time of 3-4 days
11	*E. hawkinsi*	Sheep and goats	20-25x15-23	Sub spherical	- micropyle present - triangular polar cap - Sporulation time of 5-6 days
12	*E. inetricata*	Sheep	47x32 range 39-53x27-34	Ellipsoidal	- Well developed micropyle - Sporulation time of - 3-5 days - Prepatent period of 20-27 days
13	*E. marsica*	Sheep	15.4-22.3x11.5-14.6 (mean 19.1x13.1)	Ellipsoidal	- Wall two layered, smooth, colorless to pale yellow - Shallow dome-shaped micropylar cap

					- Sporulation time of 72 hours - Prepatent period-14-16 days
14	*E. ninakohlyakimovae*	Goats	23.1x18.3 range of 20-28x15-22	Ellipsoidal sometimes ovoid	- No micropyle, no polar cap - Cyst wall smooth and transparent, slightly brownish yellow - Sporulation time- one to two days
15	***E. ovina***	Sheep	23-26x16-24 (mean of 27x20)	Ovoid or ellipsoidal	- Oocyst wall two layered with outer layer smooth, yellowish - Micropyle present covered with a micropyle

					cap - Sporulation time-2-4 days
16	*E. pallida*	Sheep, goats	12-20x8-15	Ellipsoidal	- No polar cap - Oocyst wall thin , pale yellow to yellowish green - Sporulation time of 24 hours
17	*E. parva*	Sheep and goats	16.5x14.1 Range of 12-22x10-18	Subsherical to spherical	- Oocyst wall smooth with a uniform thickness, no visible micropyle, no polar cap; pale yellow to yellowish green - Sporulation time 1-2 days

18	*E. punctata*	Sheep	21.2x17.7; range 17.8-25.1x16.2-21.1	Sub spherical to spherical	- Micropyle present - Small polar cap - Wall of oocyst covered by an even distribution of cone shaped pits - Sporulation time of 36-48 hours
19	*E. webridgensis*	Sheep	17.1-30x14.4-19	Ellipsoidal to sub spherical	- Oocyst wall 2 layered, outer layer smooth,colorless or pale yellow, inner dark - Micropyle present - Dome-shaped micropylar cap
20	*E.alijevi*	Goats	15-23x12-22	Ellipsoidal, Sub spherical,	- No micropyle

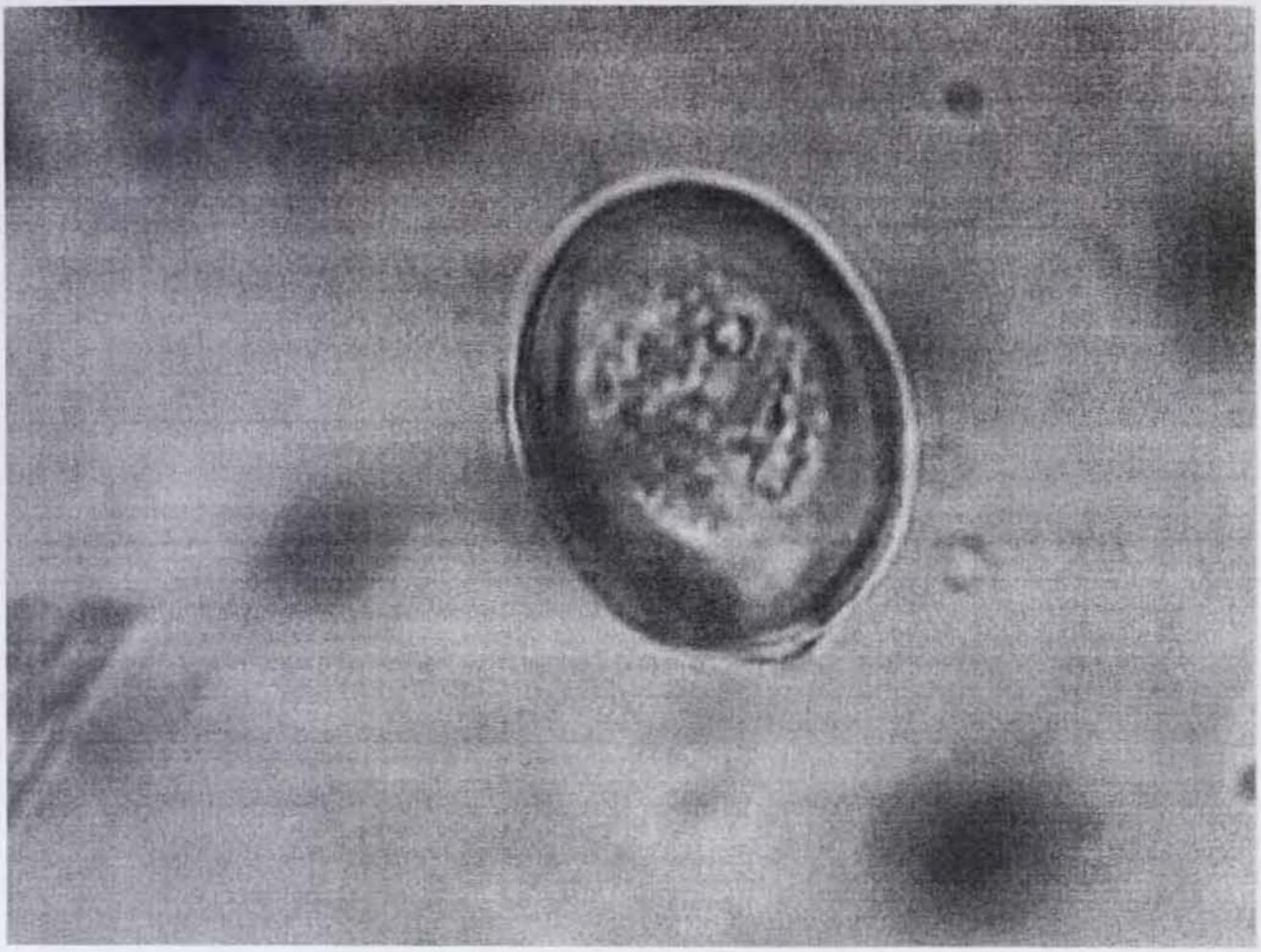
				Spherical or ovoid	cap - Sporulation time 1-5 days
21	*E.apsheronica*	Goats	24-37x18-26	Ellipsoidal	- Micropyle without polar cap - Sporulation time of 1-2 days - Prepatent period of 14-17 days
22	*E.caprina*	Goats	27-40x19-26	Ellipsoidal to slightly ovoid	- Sporulation time of2-3 days - Prepatent period of 3-6 days - Micropyle without polar cap
23	*E.caprovina*	Goats	26-36x21-28	Ellipsoidal, Subsphericalor slightly ovoid	- Sporulation time of 2-3 days - Prepatent period of 14-20 days - Micropyle without polar cap

24	*E.hirci*	Goats	17-29x14-22	Ellipsoidal to subspherica	- Micropyle with polar cap - Sporulation time of 1-3days - Prepatent period of 5-14 days
25	*E.jelchijevi*	Goats	26-37x18-26	Ellipsoidal to ovoid	- Micropyle with polar cap - Sporulation time of 2-4 days - Prepatent period of 14-17 days

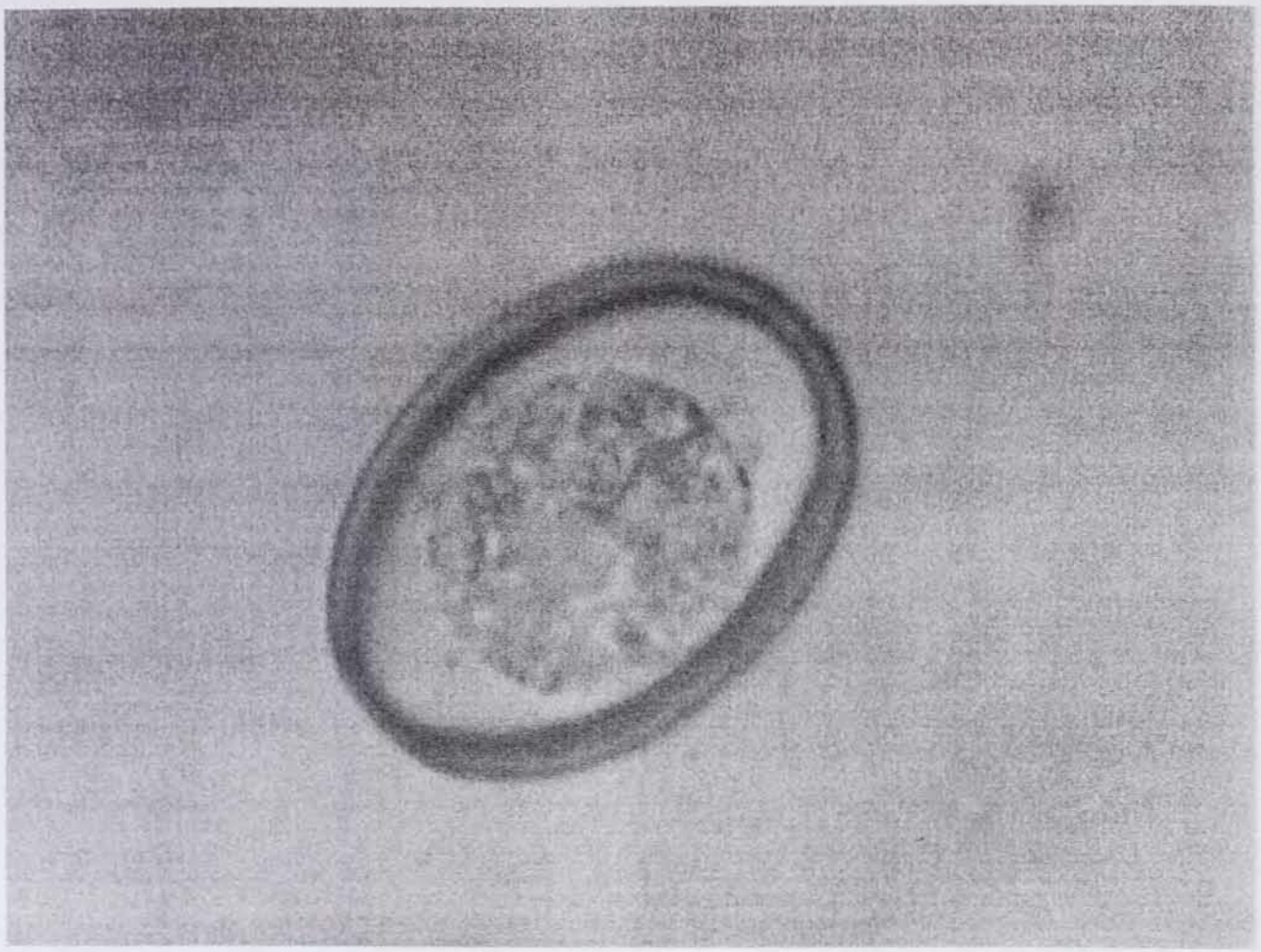
Annex 5 Recording sheet for data collection

Sample No.	Study site	Date of sampling	Animal host	Age	Sex	Parasites identified on flotation	OPG	Acid fast test	Remark

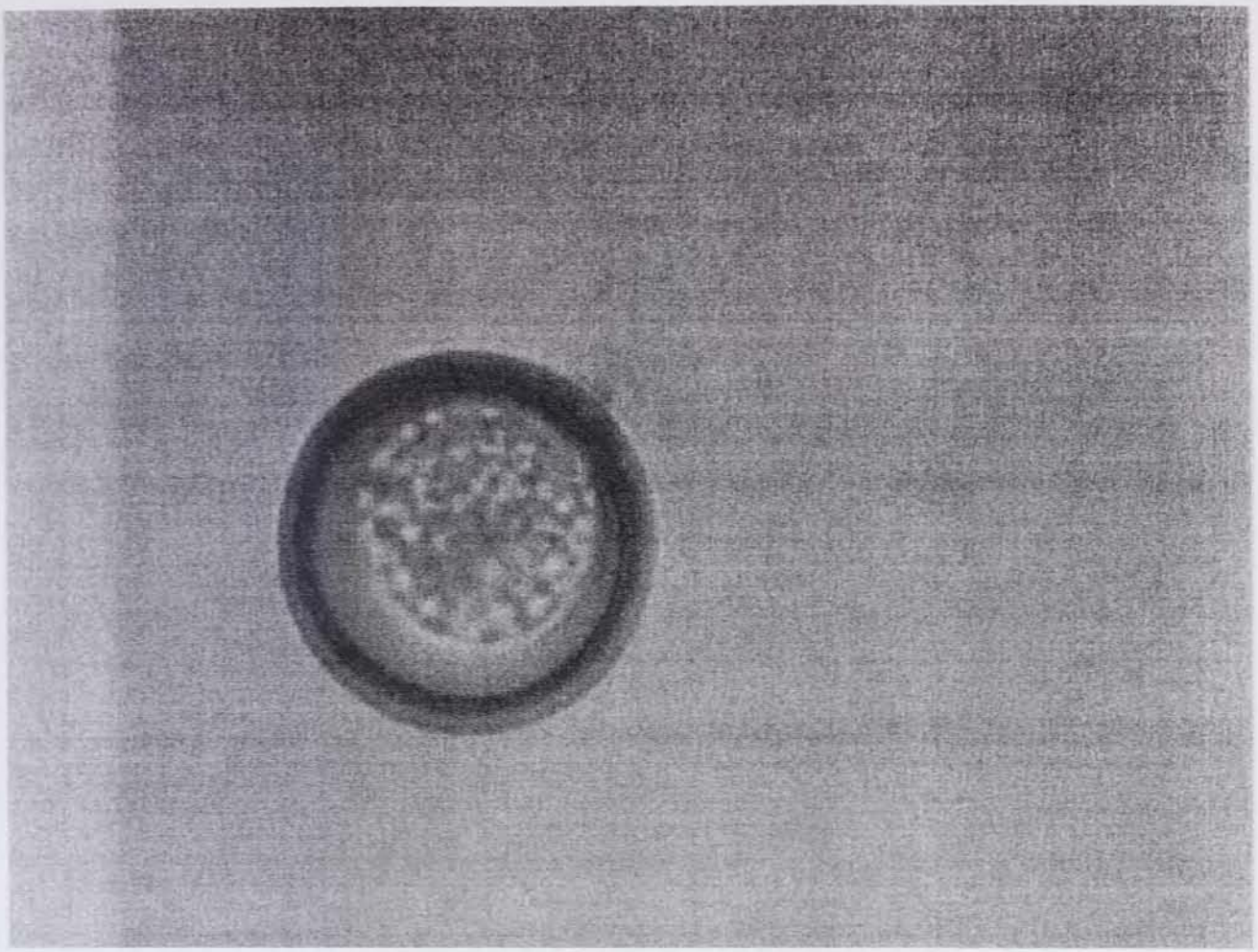
Annex 6 Some of the different *Eimeria* species identified during the study period



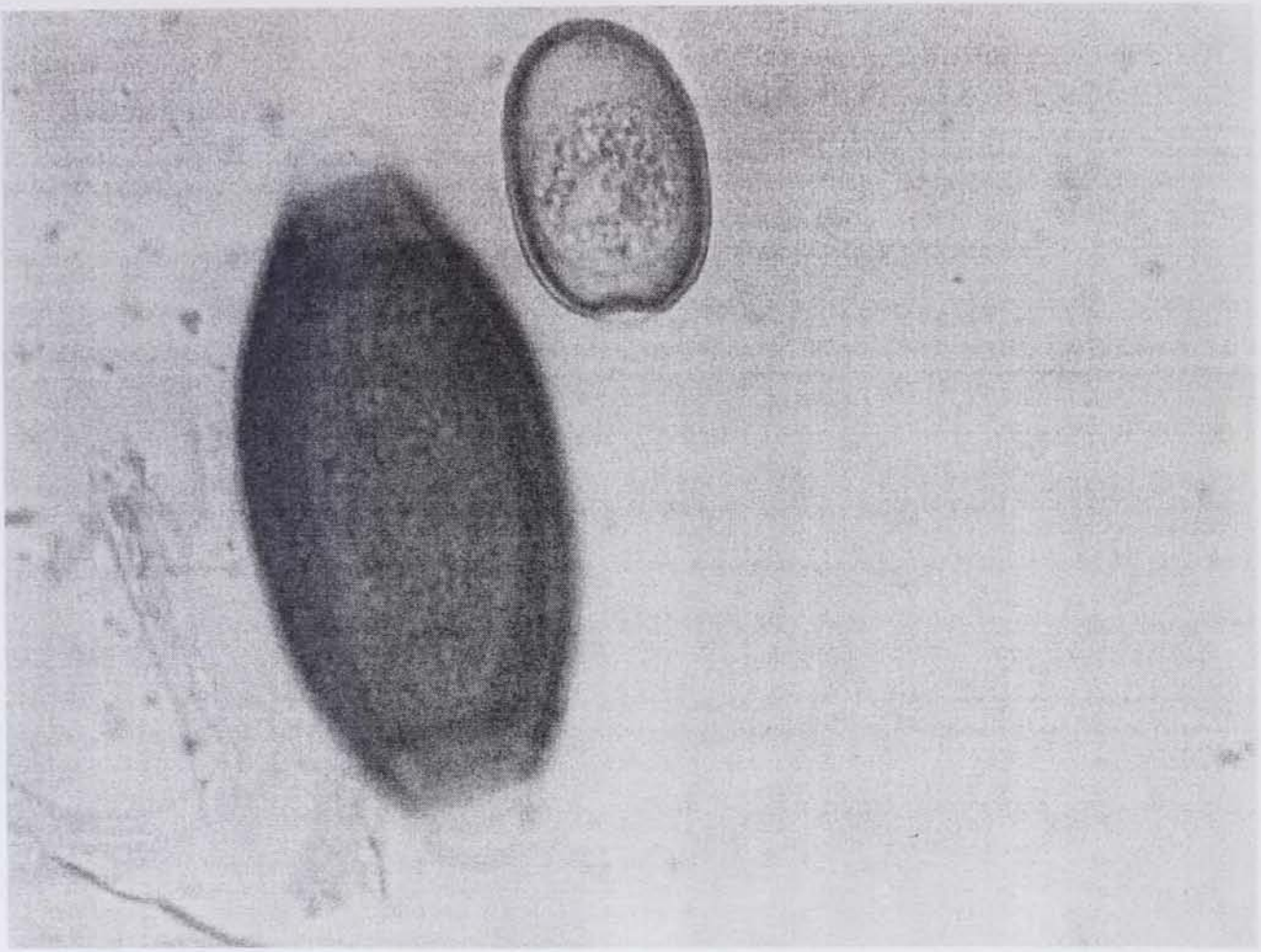
a. *Eimeria crandallis* (From sheep) 40x



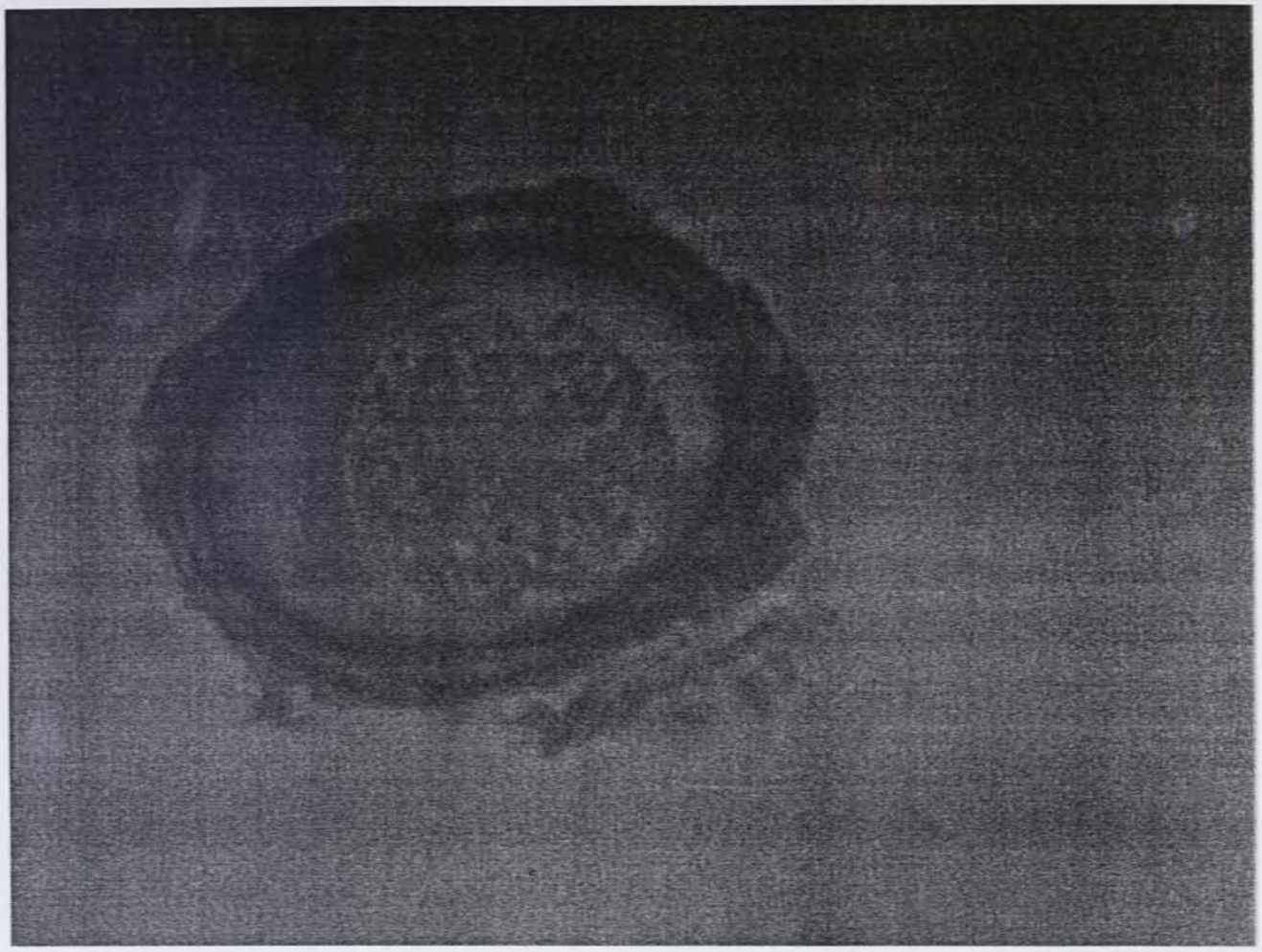
b.Eimeria webridgenesis (Sheep) 40x



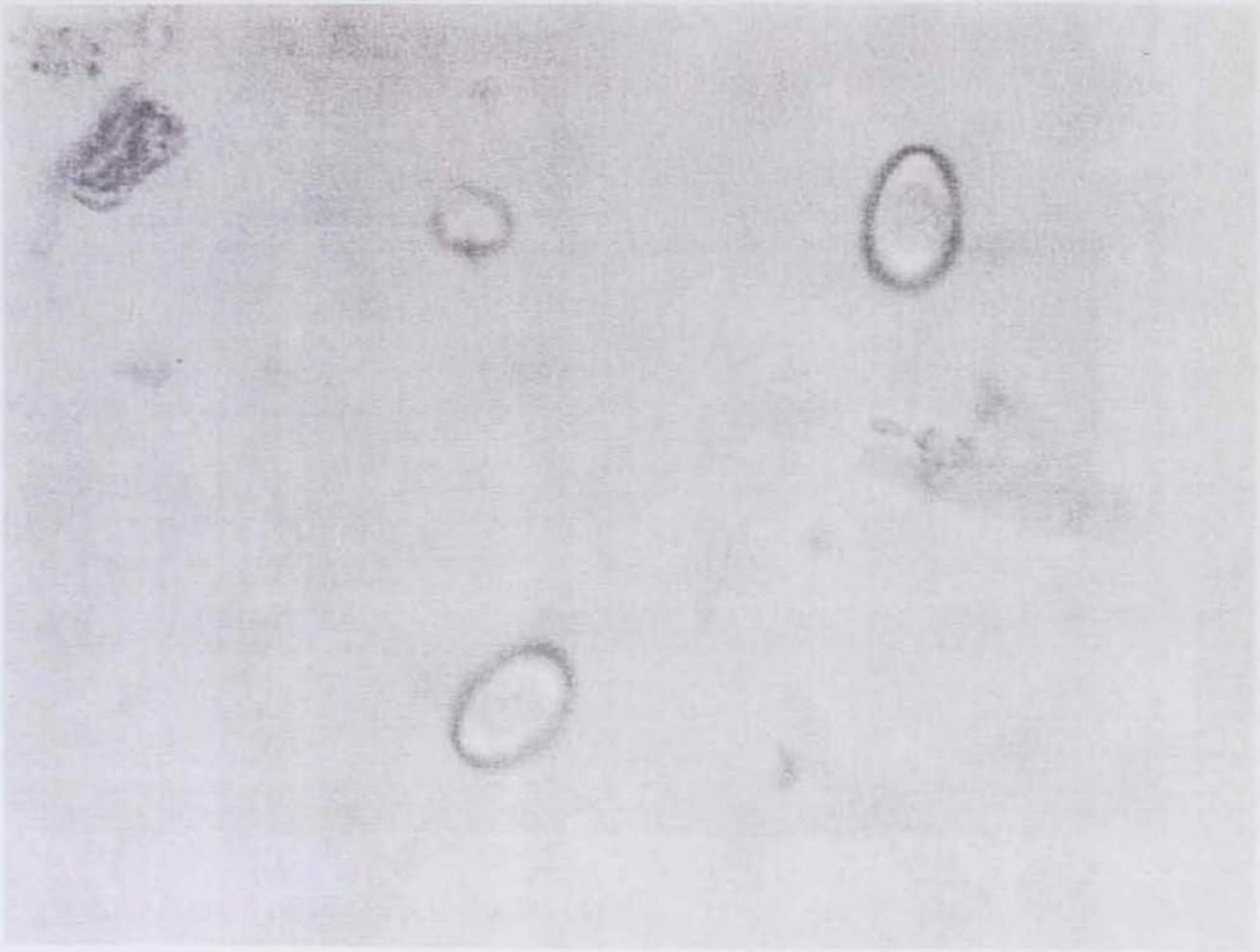
c. Eimeria parva (From sheep) 40x



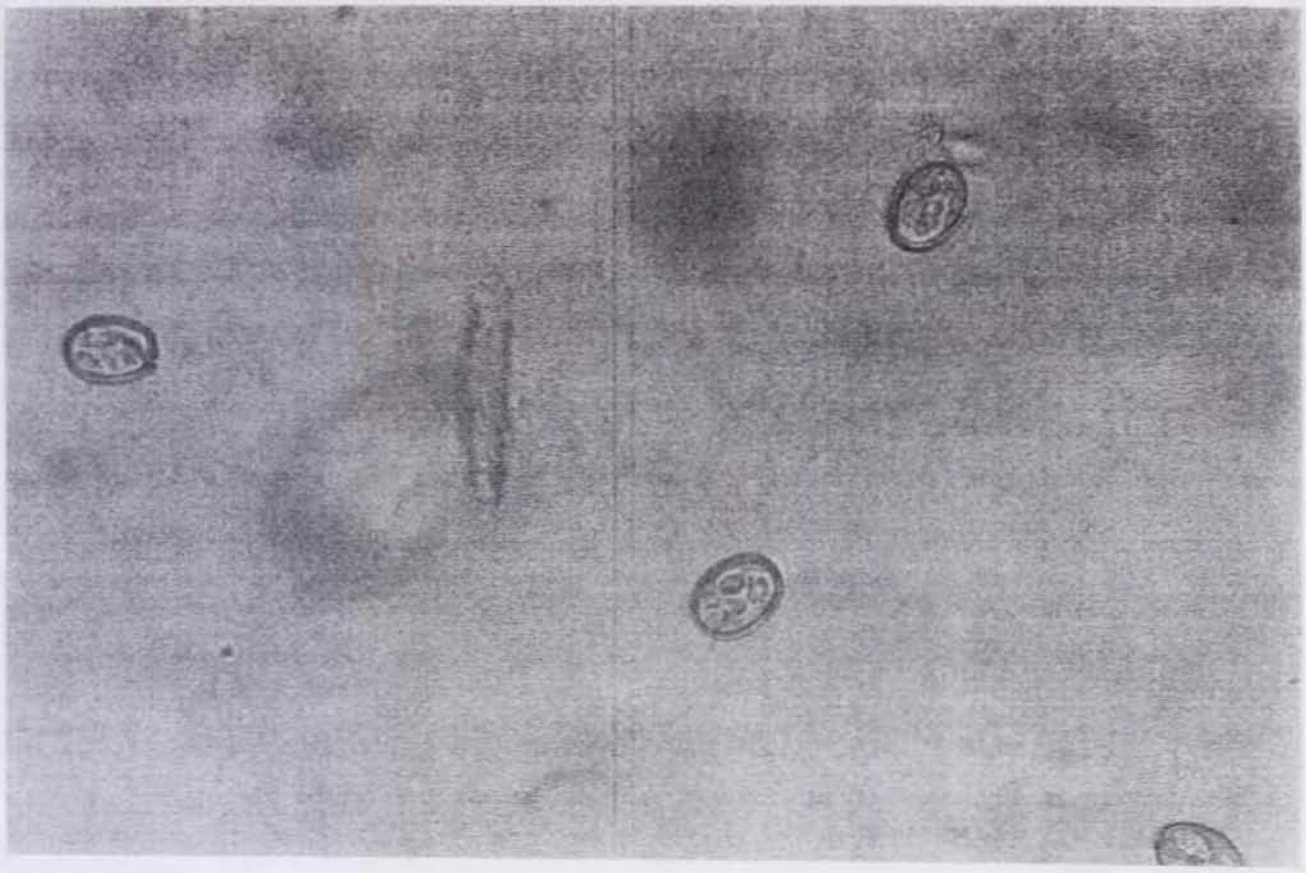
d. Eimeria arloingi (From goats) 40x



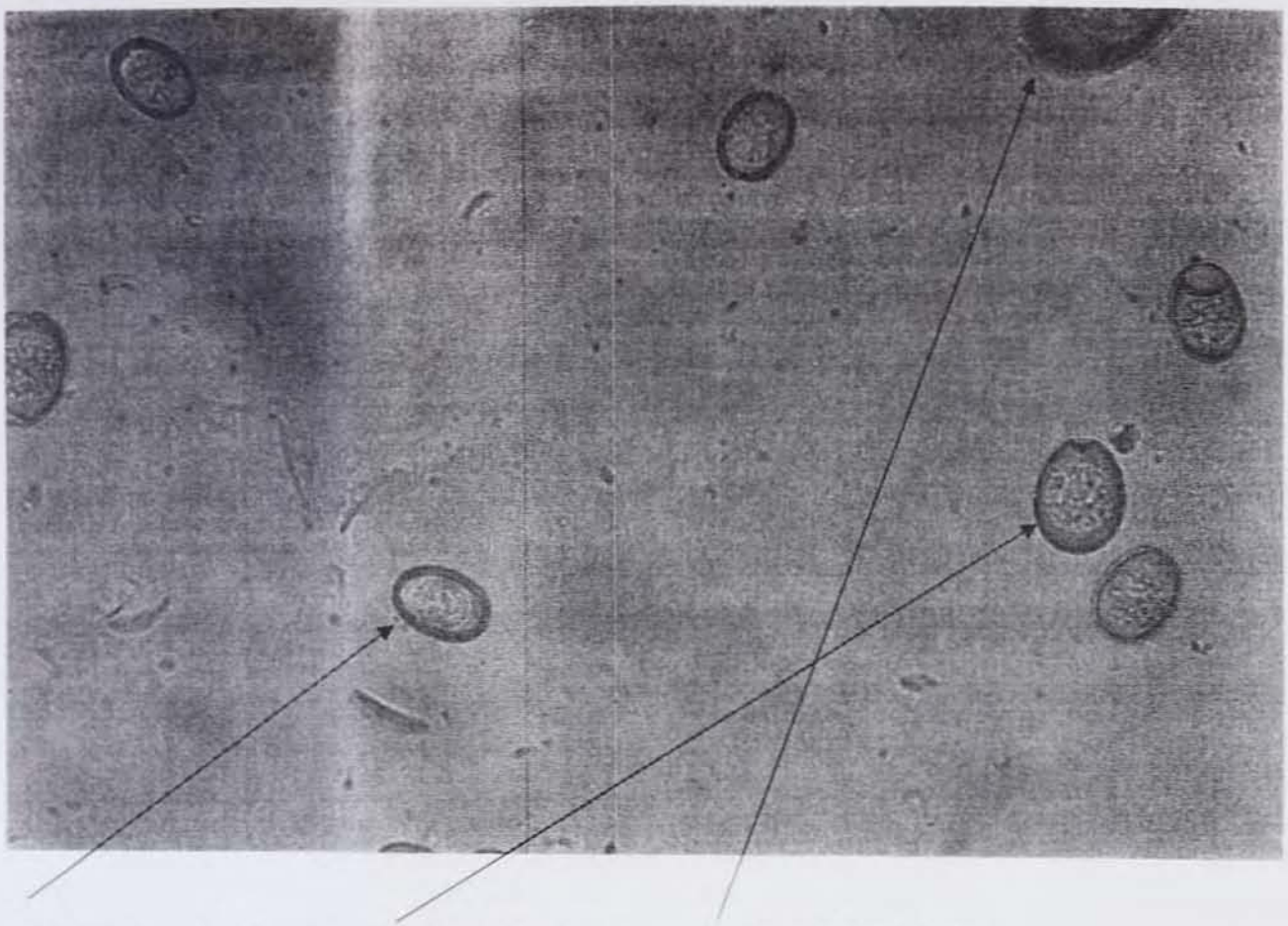
e. Eimeria intricata **(From sheep) 40x**



f. Eimeria ninakohlyakimovae (From goats) 40x



g. Sporulated oocysts of *Eimeria crandallis* *40x*



h. Eimeria webridgenesis *Eimeria faurei* *Eimeria intricata*

(Mixed infections)

Annex 7 Preparation of sheather's sugar solution according to Hendrix (1998):

- About 454g of granulated pure cane sugar (table sugar) is added to 355ml water by stirring over indirect heat (top half of a double boiler).
- After the sugar is dissolved, the solution is allowed to cool at room temperature.
- While stirring, 6ml of 40% formaldehyde solution is added to this solution to prevent mold from growing. This solution should have a specific gravity of between 1.2 to 1.25. In this range, faecal material, much of which has a specific gravity of 1.3 or greater, does not float. If the specific gravity is below the desired range, we add more reagent until the hygrometer indicates this range. If the specific gravity is above 1.25, we should add water until the proper reading is obtained.

Annex 8 Procedure for the sheather's sugar flotation technique (Hendrix, 1998):

- Using a paper cup and tongue depressor, mix approximately 2-3g of faeces with enough water to make a semisolid suspension
- Strain the mixture (faeces and water) through a double layer of cheese cloth (or two layers of single sheet gauze) or a tea strainer over a second paper cup. Use the tongue depressor to press out most of the liquid; return the solid waste to the first cup and discard.
- Pinch the rim of the second paper cup to form a pouring spout and transfer the contents into a 15ml centrifuge tube (test tube) and place the tube into the centrifuge,

remembering to counterbalance the tube with an identical tube filled to the same level with water.

- Centrifuge for 3 minutes at 400x to 650xg. For many centrifuges, this is about 1500 revolutions per minute (rpm). Decant the supernatant, which contains fats and dissolved pigments that interfere with the identification of parasite eggs, larvae or cysts.
- Add concentrated flotation solution to 1cm from the top of the tube and resuspend the sediment using a stirring action with a wooden applicator stick. Insert a rubber stopper and mix by 4 or more inversions, so that the solution is thoroughly mixed with the sediment.
- Return the tube to the centrifuge and centrifuge for 5 minutes. Without removing the tube from the centrifuge, pick up the surface film containing eggs, larvae or cysts by touching the surface gently with a wire loop (bent at a 90 degree angle) or a glass rod. Transfer the surface film to a glass microscope slide and add a cover slip. This is for a fixed angle centrifuge. If the rotor on the centrifuge is not angled, the centrifuge tube can be filled with flotation solution until a reverse meniscus is present and a cover slip can then be added; centrifuge the tube with the cover slip in place for 5 minutes. After centrifugation, lift the cover slip straight up and place it on the glass slide.
- Examine the slide under the compound microscope using the 10x objective for the identification of Eimeria oocysts and 40x objective for Cryptosporidium oocysts.

Annex 9 The procedure to determine Oocyst per gram of faeces (OPG) according to Kaufmann (1996):

1. Weigh 3g of faeces and add 42ml tap water
2. Homogenize and pour suspension through a 250μm aperture sieve, collecting the filtrate
3. Collect the filtrate, agitate and fill a test tube of 15ml volume
4. Centrifuge at 2000rpm for 2 minutes
5. Pour off the supernatant, agitate sediment and fill tube to previous level with flotation solution
6. Invert tube 6 times and remove fluid with pippet to fill both chambers of Mc Master slide quickly
7. Examine one chamber and multiply number of oocysts under one etched area by 100, or two chambers and multiply by 50 to arrive at the number of oocysts per gram (OPG) of faeces.

Annex 10 Procedure of faecal culture for oocyst sporulation(Hendrix,1998):

- When the Coccidian oocysts are found in a fresh faecal sample, place 10 to 20 g of the sample in a beaker or a paper cup and cover with about 60 ml of 2.5% potassium dichromate solution. Mix this solution thoroughly with a tongue depressor.
- Pour in to a petridish and allow incubating at room temperature for 3 to 5 days. Open the plate daily and swirl the contents gently to allow air to reach the developing oocysts.
- After incubation, centrifuge the plate's contents as indicated for sedimentation procedure
- Process the faecal sediment by the centrifugal flotation procedure to recover the oocysts, then examine microscopically. A fully sporulated oocyst of the genus Eimeria contains four sporocysts, where as a fully sporulated oocyst of the genus Isospora has two sporocysts. Examining their shape, size and the

presence or absence of a micropyle one can differentiate the species of Eimeria.

9. CURRICULUM VITAE

1. Personal information

Name: Dinka Ayana Aga
Date of birth: August 30, 1972
Place of birth: Wollega
Language skill: Afaan Oromoo, English and Amaharic
Nationality: Ethiopian
Contact address: dinka_ayana@yahoo.com

2. Educational background

September 1979- June 1984: Primary education, Walgo Aira primary school.
September 1985- June 1990: Secondary education, Lalo Aira secondary high school.
September 1991- July 1996: Addis Ababa university, Faculty of Veterinary Medicine, Debre-zeit and received DVM.

3. Work experience

October 1997- August 2001: Oromia Agricultural Development Bureau, East Wollaga adminstrative zone, Horo District Agricultural Development Office, veterinary services team leader.
September 2001- August 2002: Oromia Agricultural Development Bureau, East Wollaga administrative zone, Horo District Agricultural Development Office, head of livestock development and veterinary services department.
September 2002- August 2003: Team leader of Vet. Services in the same district.

4. Research out puts

1. Dinka Ayana (1996): Preliminary study on the prevalence of fasciolosis in small ruminants in and around Assela, Arsi administrative zone, Ethiopia, DVM thesis, FVM, Addis Ababa university.
2. Dinka Ayana (2006): Studies on Coccidiosis and Cryptosporidiosis in small ruminants at ELFORA export abattoir and in & around Debre-zeit, Ethiopia, MSc thesis, FVM, Addis Ababa University.

5. Technical papers

1. Brucellosis: From public health point of view, seminar on current topics, 1995, Addis Ababa University, FVM, Debre-zeit, Ethiopia.
2. Major protozoa infections causing diarrhea in small ruminants, seminar on current topics, 2005, Addis Ababa University, FVM, Debre-zeit, Ethiopia.

10. SIGNED DECLARATION SHEET

I, the under signed, declare that the thesis is my original work and has not been presented for a degree in any University.

Name_____

Signature_____

Date of submission_____

This thesis has been submitted for examination with my approval as a University advisor.

_____

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Studies On Coccidiosis &Cryptosporidiosis In Small Ruminants At Elfora export Abattior & In & Around Debre-Zeit, Ethiopia

Dinka Ayana