STUDY ON PREVALENCE OF POULTRY COCCIDIOSIS IN AND AROUND AMBO, WEST SHEWA ZONE, OROMIA REGIONAL STATE, ETHIOPIA

MSc Thesis

By

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MSc PROGRAM IN TROPICAL VETERINARY PARASITOLGY

JUNE, 2006

BISHOFTU, ETHIOPIA

STUDY ON PREVALENCE OF POULTRY COCCIDIOSIS IN AND AROUND AMBO, WEST SHEWA ZONE, OROMIA REGIONAL STATE, ETHIOPIA

A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical veterinary parasitology

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June, 2006

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DEDICATION

I dedicate this thesis munscript to my all family members for nursing me with affection and love and for their dedicated partnership in the success of my life.

STATEMENT OF AUTHOR

First, I declare that this thesis is my own work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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Date of Submission: 09/06/2014

ACKNOWLEDGMENTS

First and for most, blessed be my heavenly father, God, who helped, lead and strengthen me to finish this thesis successfully.

I would like to express my heartfelt thanks to my advisor, Dr. Dinka Ayana, Dean of College of Veterinary Medicine and Agriculture of Addis Ababa University for his valuable suggestion, comments and shaping my research proposal and thesis. Dr. Waktole Terfa, my co-advisor at Ambo University, takes my thanks and gratitude for his comment on the proposed title of the research from the beginning of the job, provision of materials and for his noble hearted help, guidance, cooperation and encouragement which felt me with the spirit of confidence to successfully complete this research work.

I am highly grateful to Ambo University College of Agriculture and Veterinary Sciences department of Veterinary Laboratory Technology for their financial and materials support from the beginning to the end to complete this research project.

I am highly indebted to Dr. Firaol Tamiru, instructor in Ambo University department of Veterinary Laboratory Technology; he has initiated the research topic and facilitated the process before I start the research.

My special thanks also go to the staff member of Veterinary Laboratory Technology department for their assistance during laboratory work. Had it not been for their good cooperatives spirit and technical assistance in this work, it would have not been possible to successfully complete the research work.

I would like to thanks the staff of the National Animal Health Diagnostic and Investigation Center (NAHDIC) for provision of laboratory premises and assistance during histopathological examination especially I pleasantly express my thanks to Dr.Alemnesh Woldeyes and staff of pathology department; for their technical support during the conduction of this research.

I would like to acknowledge all my family members with deep appreciation for their indispensable helps and encouragement; specifically my heartfelt thanks go to my beloved wife W/o Konjit Asheber for her sincere encouragement, her prayers and patience throughout my study.

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LIST OF ABBREVIATIONS

AARDB	Ambo agricultural and rural development bureau
ATMA	Ambo town ministry of agriculture
CSA	Central statistics authority
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and agriculture organization
ILRI	International livestock research institute
ITS1	ribosomal DNA internal transcribed spacer1
KPMRC	Kombolcha poultry multiplication research center
NAHDIC	National animal health diagnostic and investigation center
PCR	Polymerase chain reaction
RIR	Rhode Island Red
rRNA	ribosomal ribonucleic acid

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Abstract

A cross sectional study was conducted on poultry coccidiosis of local and Rhode Island breed from October 2013 to February 2014 in and around Ambo town, Ethiopia. The objectives of this study were to determine the prevalence of coccidiosis, to identify species of *Eimeria* and to assess potential risk factors(breed,age sex and manegment system). The study involved postmortem examination of chickens, mucosal scraping and parasitological examination of oocyst, gross and histopathological examination and identification of *Eimeria* species. Out of 390 examined chickens 18.7%(73/390) of them were harbor different *Eimeria* species. Statistically no significant differences $p>0.05$ ($\chi2=0.921$, $p= 0.337$) was noted between young and adult age groups. Additionally there were no significant differences between local and Rhode Island Red breed($\chi2= 0.16$, $P>0.05$), management systems (extensive and semi intensive)($\chi2= 2.245$, $P=> 0.05$ and sexes($\chi2= 3.609$, $p>0.05$). In the attempt made to identify the prevalent species of coccidian in the study area, five *Eimeria* species were identified, namely *Eimeria. tenella, Eimeria. necatrix, Eimeria. burnette*, *Eimeria. maxima* and *Eimeria. acervulina* with the prevalence of 60.27%, 15.06%, 9.6%, 1.4% and 1.4% respectively. *Eimeria. tenella* was the predominant species in the study area. In conclusion, the present study showed that coccidiosis is an important disease of poultry in the study area and further strategy needs to be implemented to reduce the loss due to coccidiosis.

Key words: Ambo, coccidiosis, *Eimeria*, poultry, prevalence

1. INTRODUCTION

The world poultry population has been estimated to be about 16.2 billion, with 71.6 % in developing countries, producing 67, 718,544 metric tons of chicken meat and 57,861,747 metric tons of hen eggs per annum (Gueye, 2005). In Africa, village poultry contributes over 70% of poultry products and 20% of animal protein intake. In East Africa, over 80% of human population live in rural areas and over 75% of these households keep indigenous chickens and Ethiopia is not exceptional from this situation (Kitalyi, 1998).

Ethiopia has large population of chickens estimated to be 48.89 million with native chickens of non descriptive breed, hybrid of chickens and exotic breed of chickens mainly kept in urban and peril-urban areas representing 96.6%, 0.55% and 2.8%, respectively(CSA, 2011). From the total population of chicken in Ethiopia, 99 % are raised under the traditional back yard management system, while 1 % is under intensive management system (Tadelle *et al.,* 2003).

Poultry production offers an opportunity to feed the fast growing human population and to provide income for resource poor farmers (CSA, 2004). Moreover, poultry in many parts of the modern world is considered as the chief source of not only cheaper protein of animal origin but also of high quality human food (Jordal *et al*., 2002). Three types of poultry production systems are identified in Ethiopia (Yami and Taddele, 1997). These are backyard poultry production system, small scale and large-scale intensive poultry production systems. The main objective of rearing chicken in all production systems is concerned with egg and meat production, for income generation and home consumption (Nasser, 1998).

In developing countries, animal production is being subjected to great pressure to satisfy the demand for animal protein required by the continued increase in human population, and also to have surplus for international trade (FAO, 1993; FAO, 1998). Among the

animal production activities, poultry sector is the fastest growing. Thus, the production of poultry protein-products has greatly expanded in many of these countries in the recent past. Nevertheless, it has been adversely affected by a variety of constraints (FAO, 1998). Among the constraints, poultry diseases continue to play the major central role in hampering its development (FAO, 1998; Rushton *et al.*, 1999). In Ethiopia, poultry production has been hindered by different prevalent diseases from which Newcastle disease, coccidiosis, salmonellosis and chronic respiratory disease are the important ones (Abebe *et al*., 1997).

Among the infectious diseases of poultry, cocccidiosis is the major parasitic disease. Poultry coccidiosis is an economically important disease in chicken caused by the intracellular protozoa parasite of *Eimeria* species in the genus *Eimeria* family *Eimeridae* order *Eucoccidiorida* and phylum *Apicomplexa* (Taylor *et al*., 2007). Infection by coccidia in sufficient number to produce clinical manifestations of disease is called coccidiosis (Charlton, 2006; Conway and Mckenzie, 2007). Though nine species of *Eimeria* have been identified as causative agents of poultry coccidiosis, only seven of them have been reported to be pathogenic (Kahn, 2008). *Emeria tenella* (*E. tenella*) and *Emeria necatrix* (*E. necatrix*) are the most pathogenic species. *Emeria arcevulina* (*E. acervulina*), *Emeria maxima* (*E. maxima*) and *Emeria mivati* (*E. mivati*) are common and slightly too moderately pathogenic while *Emeria brunetti* (*E. brunetti)* is uncommon but pathogenic when it does occur. *Emeria mitis* (*E. mitis*), *Emeria praecox* (*E. praecox*) and *Emeria hagani* (*E. hagani*) are relatively non-pathogenic species (Soulsby, 1982; Lillehoj and Trout, 1993).

The disease is endemic in most of the tropical and subtropical regions where ecological and management conditions favour an all-year round development and propagation of the causal agent (Obasi *et al*., 2006). In Ethiopia, poultry coccidiosis caused by(*E. acervulina, E. necatrix, E. maxima* and *E. tenella*, is endemic in all parts of the country and affects mainly young growing birds (Safari *et al*., 2004).

Coccidiosis remains one of the major disease problems of poultry in spite of advances made in prevention and control through chemotherapy, management and nutrition (Graat *et al*., 1996). In all parts of the world where confinement rearing is practiced, coccidiosis represents a major disease problem demanding the attention of poultry producers, feed manufactures, and poultry disease experts (Reid, 1978).

The occurrence of clinical coccidiosis is directly related to the number of sporulated oocysts ingested by poultry at one time, the pathogenicity of the *Eimeria* species, the age of the infected chicken and the management system (Reid, 1990). The first and most frequent symptom is at the beginning yellow diarrhea. As the disease progresses, because of the blood in feces, feces are red or resemble the color of chocolate (Jordan, 1990). The feathers around the cloacae are covered with bloody deposits. Feces are stained with blood. Birds that survive first few days of the infection, can survive the next 10 to 15 days. During that time, birds are thirsty and rapidly lose weight (Calnek, 1997). Symptoms of the disease start to appear at the time when the second generation of shizonts starts rapidly to replicate, grow, mature and release the second generation of merozoits. Second generation of merozoits causes inflammation of the sub epithelial mucus, desquamation of the epithelia and capillary rupture in the caecum wall. As a consequence, bloody diarrhea occurs (Jordan, 1990). *Emeria tenella* causes moderate to severe cecal lesions, sometimes death. The birds become depressed, have ruffled feathers, the wings droop, have diarrhea and tend to huddle. Food and water consumption usually decreases and may become emaciated and dehydrated. Laying hens will experience a reduction in rate of egg production. Cecal coccidiosis may produce bloody droppings and anemia (Whitmarsh, 1997; Lillehoj and Trout, 1993). In large population of chicken kept confined together, the risk of acquiring sufficient dose of oocysts is more likely to occur and the risk factor is very high for young chicken than old age groups, which develop immunity from pre-exposures (Becker, 1962). Losses due to mortality following a severe outbreak may be devastating and incidence rates as high as 80% were observed to occur in the form of an outbreak in Ethiopia (Alamargot, 1987). However, losses due to morbidity may be even more costly without the producers being aware that their flocks having any disease problem (Alamargot, 1987).

World-wide losses to poultry industry due to coccidiosis have been estimated about 800 million $ annually (Mohmad and Hidayatullah, 2013), which may be considered as a reason for devising improved control strategies. The estimates include the costs of prophylactic in feed medication for broilers and broiler breeders, alternative treatments (e.g. with amprolium) if the medications fail, and losses due to mortality, morbidity, and poor feed conversion of birds that survive out breaks. Quantitative losses due to coccidiosis in Ethiopia are not well documented, but (Kinung'hi *et al.* 2004) has reported that coccidiosis contributes to 8.4% loss in profit in large-scale farms and 11.86% loss in profit in small-scale farms.

Poultry is the most important animal species in and around ambo town both for nutritional value they contribute and cash income generation than other animals since they are the main resources especially for poor families. Additionally, poultry raising system is now becoming more intensified than the past in which coccidiosis is the area of focus. Despite several researches have been undertaken on poultry coccidiosis in different parts of our country (Ashenafi, 2000; Methusela, 2001; Lobago *et al*., 2003; Safari *et al*., 2004; Gari *et al*., 2008; Mersha *et al*., 2009; Abadi *et al*, 2012; Dinka and Yakob, 2012), the disease is still a major problem demanding much research and investigation. Regarding poultry coccidiosis in West Shewa Zone generally in and around Ambo town specifically, information is scant both on local and cross breed chickens.

Therefore, the objectives of this study were

- To determine prevalence of poultry coccidiosis in and around Ambo
- Identify the circulating Eimeria species
- To identify the associated risk factors of coccidiosis

2. LITERATURE REVIEW

2.1 General description on poultry coccidiosis

Coccidiosis is a disease of poultry caused by a protozoan parasite. This parasite lives and multiplies in the intestinal tract and causes tissue damage. This damage can interfere with the food digestion and nutrient absorption, as well as causing dehydration and blood loss. The tissue damage can also expose the bird to bacterial infections, like *Clostridium* and *Salmonella*. Diseases that suppress the bird's immune system may act with coccidiosis to produce a more severe problem. For example, Marek's Disease may interfere with the development of coccidiosis immunity and Infectious Bursal Disease may exacerbate a coccidia infection (Julie, 1999).

2.2 Etiology

Poultry coccidiosis is caused by a protozoan parasite known as *Eimeria*. A numbers of *Eimeria* species have been recorded from poultry (Table 1) which are affecting a particular part of the intestinal tract (McDougald, 1998).

Table 1:- Species of *Eimeria* with their predilection site in the host.

Species	Site of lesions
E. tenella	Caecum
E. acervulina	Duodenal loop
E. necatrix	Mid gut
E. maxima	Mid gut
E. hagani	Anterior gut
E. mivati	Duodenal loop to rectum and caecum
E. praecox	Anterior gut
E. mitis	Anterior gut
E. brunette	Lower intestine

Source: (Foreyt, 2001)

Each species is host specific and able to produce specific immunity in the bird, but there is no cross immunity between species. Species of *Eimeria* that is pathogenic and economic importance are *Eimeria tenella*, which causes the caecal or bloody type of coccidiosis, *E. necatrix*, which causes bloody intestinal coccidiosis, and *E. acervulina* and *E. maxima*, which cause chronic intestinal coccidiosis.

2.3. Morphology

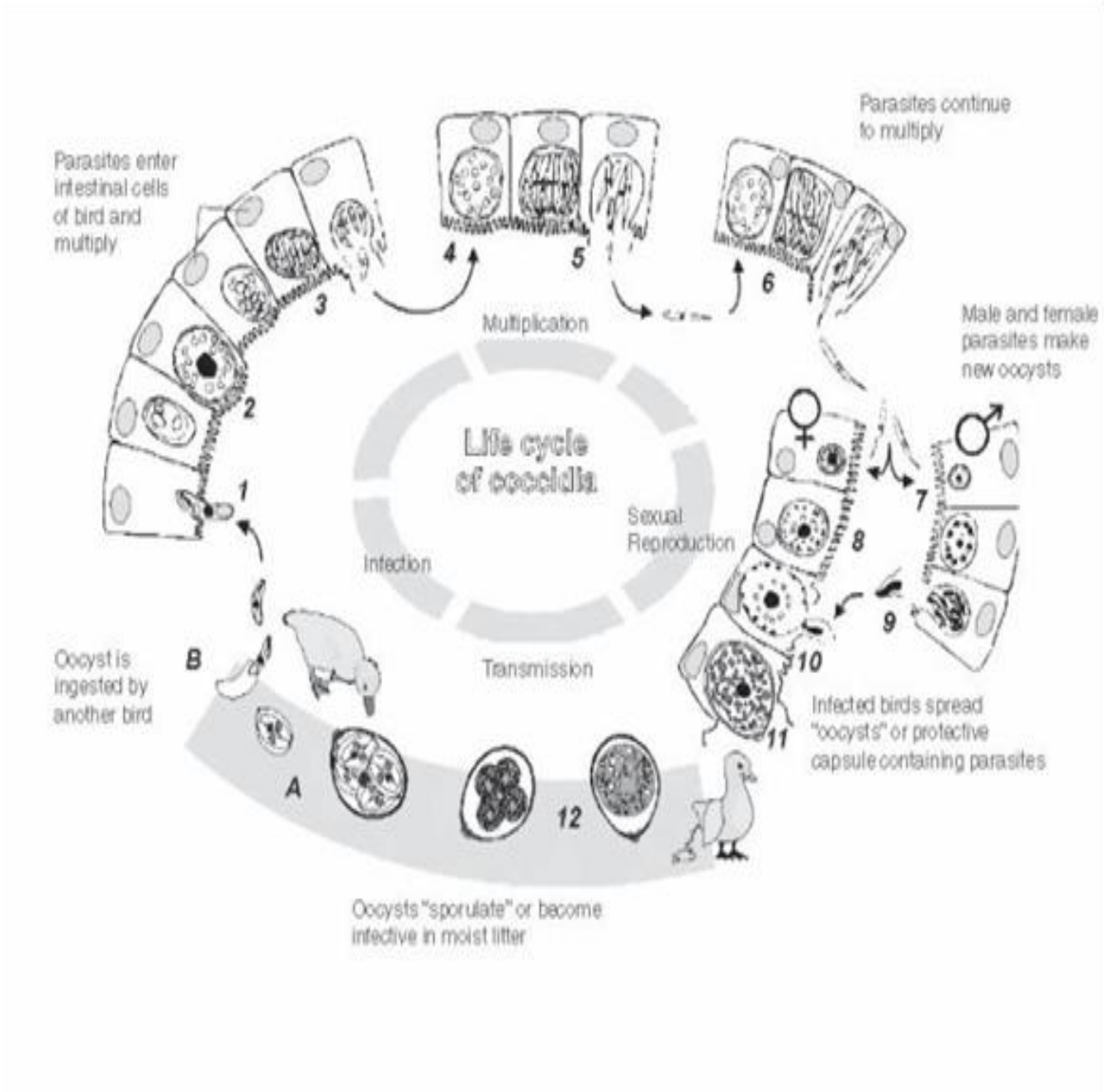
Majority of *Eimeria* oocysts have ovoid shape. *Eimeria maxima* (30.5 x 20.7μm) is the largest while *Eimeria mivati* (15.6 x13.4μm) and *Eimeria mitis* (15.6 x 14.2μm) are the smallest as compared to other species of *Eimeria*. Oocyst size, shape and color are helpful in identification of *Eimeria* species. *E. tennela, E. maxima, E. acervulina, E. hagani* and *E. burnetti* are ovoid while *E. necatrix* is oblong (Reid, 1978). Other characteristics that is useful in species identification includes : zone of intestine parasitized, nature of macroscopic lesions, minimum sporulation time, minimum prepatent period, schizonts size and area in which it develops, location of the parasite within the epithelial cells and cross-immunity trails(Reid, 1978).

2.4. Lifecycle

The life cycle of all *Eimeria* species involves two or more generation of an asexual development known as shizogony, followed by a sexual phase formed by gametogony which results in the formation of oocyst (Kaufman, 1999). The infective stage, sporulated oocyst, is ingested and the action of mechanical and chemical factors in the gut (bile salt and trypsin) leads to the release of sporocysts and then sporozoites in the duodenal lumen. The sporozoites invade the mucosa sometimes passing down the whole length of the alimentary tract before doing so. Then follow phases of intracellular growth and asexual multiplication with periodic release of merozoites entering in to the sexual phase of the life cycle known as gametogenesis (Jones *et al*, 1996). These merozoites invade cells and develop in to either macro- gametes or micro- gametes. The former gives rise to a single macrogamete whereas the male gametocyte matures and ruptures, releasing a

large number of minute biflagellate micro-gametes (Fig 1). The micro-gametocyte grows to form a micro-gamete. A thickened wall forms around the macro-gamete, forming a zygote when the macro gamete is fertilized by microgamete. This stage is the young or immature oocyst (Conway and Mckenzie, 2007).

Figure 1: The different stages of Eimeria in and outside intestinal cells



(Source: Fanatico, 2006)

An important feature is that the life cycle is quite rapid with a prepatent period of about 4-5 days (which varies slightly with species) and involves colossal multiplication. The degree varies with species but optimally may result in hundreds of thousands or even millions of Oocyst produced from one ingested Oocyst (Jordan *et al*., 2002).

The biochemical and genetic mechanisms that control the development of *Eimeria* species within host cells are not known. However, through study of precocious and drug-resistant lines of *E. tenella* (Shirley and Harvey. 2000), two linkage groups associated with these traits have been identified and mapped to parasite chromosomes 1 and 2. This information may help identify other genetic loci involved in regulating the life cycle of *E. tenella*. Other researchers (Ouarzane *et al,* 1998) have recently identified a gene (ets3a) whose expression is developmentally regulated and which may be important in controlling the life cycle of *E. tenella*.

2.5. Epidemiology

Distribution and prevalence of coccidiosis is influenced by several factors: high animal density cramped on a small space, high air temperature, high relative humidity, different (especially different age) categories of birds at same place, feed change, quality of feed, as well as all other factors that compromise resistance to the disease and general health status of the birds. Onset of the disease depends on the age of the bird at the time of the first infection and number of passages of the infection (for one passage to be completed it is required 10 days), as well as on ability of the bird to develop proper specific immune response (*Hofstad,* 1984).

It is impossible under farming conditions to produce a coccidia free environment (Jordan *et al*., 2002). Birds get oocyst from food or water contaminated by faeces of infected animals. Infected chickens shed oocyst for several days or weeks. Oocyst sporulated with in two days under the proper condition and become infective. Chickens pick them up by pecking on the ground or in litter used for bedding in the house (Fanatico, 2006). Coccidial oocysts are normally introduced in to new facilities through contaminated

equipment or vehicles coming from other poultry operations (Conway and Mckenzie, 2007).

Oocysts are distributed within the poultry building, inside and outside the house by invertebrates and vermin whilst mechanical ventilation systems serve to scatter the oocysts outside the house. Fecal contamination of vehicles and personnel can spread the infection to other farms (Taylor *et al*, 2007). Peoples are important vectors of coccidian (Charlton, 2006) in disseminating oocysts, which could be carried over by manure clinging to shoes or by utensils carried about from one pen to the other. Flies, beetles, cockroaches, rodents, pets and mild birds have also been incriminated as mechanical vector (Reid, 1978).

2.5.1. Agent related risk factors of coccidial infection in poultry

The occurrence of poultry coccidiosis is dependent on both the species of *Eimeria* and the size of the infecting dose of oocysts. Due to the short prepatent period of the parasite and its high biotic potential, the number of oocysts in the litter rises rapidly (Jordan *et al*., 2002). Poultry coccidia have high capacity to reproduce within the host; this leads to a rapid increase to Success and the subsequent high level of the parasite within the susceptible host and subsequently high level of contamination of the environment (Urquhart *et al.*, 1987).

Other important factor is the resistance of sporulated coccidian oocysts to harsh environmental conditions and hence their viability outside the host is crucial regarding the course of the disease and lack of cross immunity between species of *Eimeria* predisposes birds to infection and disease outbreaks caused by different species (Jeurissen, 1996; Yun *et al*., 2000).

2.5.2. Host related risk factors

Coccidiosis is usually a disease of young birds, but birds can be infected at any time, if never exposed before. Coccidia populations take time to build dangerous levels, therefore, outbreaks usually occur when birds are between 3 and 8 weeks of age (Fanatico, 2006). High animal density cramped on a small space, age of the bird at the time of the first infection and number of passages of the infection as well as on ability of the bird to develop proper specific immune response (*Hofstad,* 1984).

2.5.3. Environmental and management related risk factors

Management of poultry houses plays a momentous function in the spread of coccidiosis because coccidial oocysts are omnipresent and are easily spread in the poultry house environment. Further, owing to their high reproduction potential, it is very complex to keep chickens coccidia free, especially under current intensive rearing conditions (Adhikari *et al.,* 2008). Prevalence varied by management and did not vary by flock size (Hadipour *et al*., 2011) while bad management, such as wet litter that encourages oocyst sporulation, contaminated drinkers and feeders, bad ventilation, and high stocking density, can worsen the clinical signs (Al-Quraishy *et al*., 2009). High temperatures and humidity encountered in overstocked feedlots, pens containing straw bedding, or in irrigated pastures, are favorable for the survival of oocysts and therefore higher infection rates compared to extensive farming systems (Vorster and Mapham, 2012). Sporulation takes one to two days under optimum conditions and oocysts fail to sporulate under adverse conditions. Optimum conditions are temperatures between 25 and 32^{o}C with plenty of moisture. Unfavorable conditions are cool temperatures (below 10^{0}C) and dry conditions, exposure to temperatures of 45-50^{0}C for about one day or short-term exposure to temperature above 56^{0}C are lethal to oocysts. When oocysts are sporulated they are very resistant to adverse environmental conditions but not freezing temperature and may survive on the pastures until the next season which then act as a source of infection when climatic conditions become favorable.

2.6. Pathology and clinical finding

The infectious forms of the causative agent are oocysts in the form of spores. Infection is by oral route, with contaminated feed and/or water. After ingestion, infectious oocysts excyst, liberating the infective form: the sporozoit. Sporozoit infect epithelial cells of the intestine. Transfer of the sporozoits up to the locus of the primary lesion is with the help of intraepithelial lymphocytes (Lawn and Rose, 1982; Daszak, 1999). The pathogenicity of coccidia depends largely on the successful replication of developing parasites inside the host. The pathogenic process starts during shizogonic phase of the parasite development. The pathogenic process during the first generation of shizonts is negligible. However, the most pathological stadium is during the second generation of shizonts. Their development, deep in the cells, results in inflammation, mucus desquamation, capillary rupture and haemorrhagiae. This stadium of the disease is accompanied with severe clinical symptoms. In this stadium, possible outcome could be death of the bird. Death is a consequence of haemorrhagiae (bird can lose 60 to 80 percent of the blood volume), toxemia or as a consequence of gangrene or rupture of the intestinal wall.

Pathogenesis is influenced by host genetics, nutritional factors, concurrent disease, and strain of the coccidium. *Eimeria necatrix* and *E. tenella* are the most pathogenic in chickens because schizogony occurs in the lamina propria and crypts of epithelium of the small intestine and ceca, respectively, and causes extensive hemorrhage. Most species develop in epithelial cells lining the villi (Kahn, 2005). Coccidiosis goes hand in hand with gut disease, because it damages the gut and allows bacteria to enter and cause secondary infections (Fanatico, 2006).

Clinical signs are associated with tissue destruction from the release of the merozoites and mature oocysts from the mucosal surface during the last generations of merogony and throughout gametogony. In severe infections, much of the mucosal epithelium is sloughed off and nutrient absorption is compromised (Jeurissen *et al.*, 1996; McDougald and Reid, 1997; Yun *et al.*, 2000). The first and most frequent symptom is at the beginning yellow diarrhea. As the disease progresses, because of the blood in feces, feces

are red or resemble the color of chocolate. The feathers around the cloacae are covered with bloody deposits. Feces are stained with blood. Birds that survive first few days of the infection, can survive the next 10 to 15 days. During that time, birds are thirsty and rapidly lose weight. Symptoms of the disease start to appear at the time when the second generation of shizonts starts rapidly to replicate, grow, mature and release the second generation of merozoits.

2.7. Necropsy findings

Coccidiosis causes a thickening of the intestines which make them feel like sausage. There may be light colored spots on the surface of the gut (Table 2), and inside the gut hemorrhages and streaks (Fanatico, 2006). The type and locations of lesions in the gut indicates the species of *Eimeria*. *Eimeria acervulina* affects the upper parts of the small intestines, you may see small red spots and white bands on it; *E. maxima* affect the entire small intestine; the intestine looks watery and in later stages have blood and mucus. The intestine may look thickened and ballooned with red pinpoint lesions. *Eimeria tenella* affects the blind sacks of the gut. The intestine may be filled with blood and pus and turn in to a solid core (Fanatico, 2006). Histopathologically, the wall of the gut is thickened indicating retention of fluid (edema). There may be blood in the lumen of the gut indicating blood loss (hemorrhage), or merely retention of an excessive amount of blood in the tissue (hyperemia) there is also infiltration with various body reaction and the development of immune response (Marquardt *et al*., 2000).

Table 2: Some characteristic lesions of *Eimeria* infection during post mortem examination

Species	Location	Lesion
E.tenella	Caeca	Severe hemorrhage with white red spots in wall of intestine
E. necatrix	Middle intestine	Severe hemorrhage with mucoid discharge whitish and red spot in wall of intestine
E. brunette	Lower half of intestine	thin walled intestine, mucoid on necrotic discharge, distension of intestine
E. maxima	Middle intestine	Distended intestine with hemorrhage spots, mucoid discharge
E. acervulina	Upper intestine	Whitish spots on wall on serous surface hemorrhage streak and whitish lesions on intestinal surface, mucoid enteritis
E. praecox	Duodenum	No lesion but slightly hemorrhagic appearance on intestinal surface of duodenum slight mucoid discharge.

Source: (Saxema *et al*., 1998).

2.8. Diagnosis

Diagnosis of coccidiosis in chicken is best accomplished by postmortem examination of representative number of birds. Diagnosis by fecal examination may lead to quite erroneous results (Soulsby, 1982). In some instances the major pathology is produced before oocysts are shed in the feces (e.g. *E. tenella*) and, conversely, the presence of large number of oocysts may not necessarily indicates a serious pathogenic condition. Thus, with *E. acervulina*, which has a high biotic potential, comparatively larger numbers of

oocysts are shed than, for example, with *E. necatrix*. Furthermore, the accurate identification of the oocysts of various poultry coccidia is not easy (Soulsby, 1982).

2.8.1. Detection of Oocyst in feces

Oocysts in faeces of infected birds can be detected using floatation methods with saturated salt or sugar solution while this method is not reliable for diagnosis of coccidiosis. It can be a useful indicator of subclinical infection. Concentration floatation technique is used for the collection of *Eimeria* oocysts from intestinal content of chickens**.** *Eimeria* oocysts isolation depends on the measurements of oocysts by using a calibrated ocular micrometer at 400x magnification and location and characteristics of intestinal lesion, oocyst morphology and sporulation time of Eimeria species (Conway and Mckenzie ,1997).

2.8.2. Examination of gross lesions

The characteristics of the observed lesions such as its location on the intestinal tract, its appearance and severity, the nature of intestinal contents and other associated gross change can be useful in establishing a diagnosis (Conway and Mckenzie, 2007). The procedures for killing birds and techniques for postmortem examination are based on the technique discussed by (Zander, 1978). The entire length of the external serosal surface of the digestive tract from the gizzard to the lower rectum needs to be examined under strong light. In examining the serosal surface a search should be made for whitish plaques or petechiae. Whitish streaks or rounded colonies of oocysts in the duodenal area often indicate *E. acervulina* or *E. mivati*. In the mid gut area on both sides of the yolk sac diverticulum, whitish plaques may be produced by colonies of *E. necatrix* schizonts (Conway and McKenzie, 1991).

While cutting, watch for thickened areas indicating parasitic invasion of the mucosa or submucosa. Presence of mucus, blood, casts, or cores and presence of cheesy coagulation necrosis should be noted. Presence of blood in the caeca suggests a diagnosis of *E.*

tenella. But bleeding may originate from the more anterior zones of the intestine and moving to the cecum may led to a misdiagnosis the case of *E. necatrix* as *E. tenella* infection. As differential diagnosis histomoniasis, Hemorrhagic Syndrome and ulcerative and necrotic enteritis may also produce somewhat similar gross lesions (Reid, 1978).

The lesion produced by *E. tenella* is found mostly in the caeca. Lesion scoring is a technique developed to provide a numerical ranking of gross lesions caused by coccidian. The entire gastrointestinal tract is removed unbroken from the bird. The gizzard and the rectum are left attached for orientation to locate the lesion observed in various parts of the intestine. The lesions are scored 1upto 4 based on the key identification characteristics discussed by (Conway and McKenzie, 1991).

2.8.3. Serological and molecular diagnosis

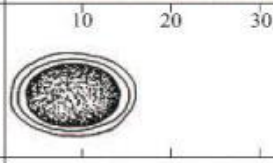
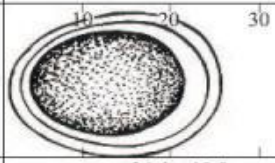
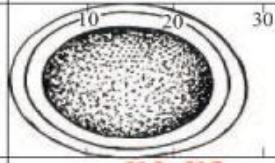
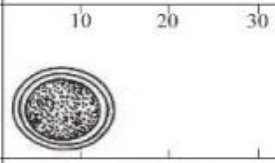
During recent years, there have been significant advances in the development of molecular-diagnostic tools. Several polymerase chain reaction (PCR) based assays targeting different regions of the *Eimeria* genome have been described, such as the 5S ribosomal ribo nueclic acid (rRNA) (the small subunit rRNA the sporozoite antigen gene EASZ240/160 (Molloy *et al* ,1998) ,internal transcribed spancer (ITS-1) (Schnitzler *et al* ,1999) and ITS-2 (Lien *et al* 2007) genomic regions. Since the ITS regions are less conserved than the rRNA genes, detecting variations in this region of DNA sequence, makes the design of primers straightforward and reduces the risk of cross reactions among different species (Holmdahl and Mattsson, 1996). Apart from an accurate identification of *Eimeria* species, molecular methods can also be helpful in epidemiological study of the parasite, an aspect that has been less investigated to date.
The application of real-time PCR to both detect and quantify species would be particularly useful for species complexes like *Eimeria* in chickens. The technique has exceptional sensitivity and, unlike traditional PCR assays, can be quantified. In 2006 two papers were published that applied real-time PCR technology to follow infection of *E. maxima* (Blake *et al*, 2006) and *E. acervulina* (Swinkels *et al.*, 2006) in laboratory

infected birds. Both of these studies were interested in studying host immunity rather than field diagnostics.

ELISAs detect antibodies in blood (sera) samples and can be designed so that they detect species specific antibodies or antibodies that recognise a range of species. Since they detect antibodies rather than the parasites themselves, ELISAs have the advantage that they are able to identify the species to which chickens have developed an immune response even when the parasites are no longer present. They also provide the ability to assess immune responses following vaccination. ELISAs are high throughput, cheap assays that would allow rapid screening of large numbers of birds. Since work began on a species-specific ELISA for *E. necatrix* has been developed by researchers in Japan (Onaga *et al.,* 2005). Their test uses a recombinant antigen, so it is specific to *E. necatrix*.

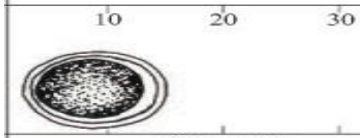
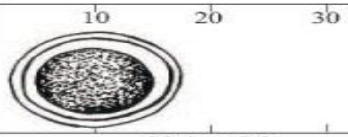
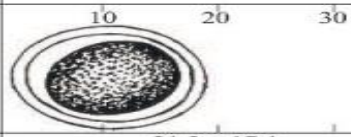
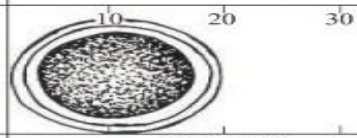
Generally the following characteristics are useful in Differential identification of each species of poultry Eimeria as zone of intestine parasitized, gross appearance of the lesion, oocyst morphology, minimum sporulation time, schizont size and location of development, location of parasite in the host intestinal epithelium and cross-immunization and they are illustrated in (Fig 2).

TABLE 1.1. Differential characteristics for eight species of chicken coccidia

	CHARACTERISTICS	*E. acervulina*	*E. brunetti*	*E. maxima*	*E. mivati*
MACROSCOPIC LESIONS	ZONE PARASITIZED				
	MACROSCOPIC LESIONS	light infection: whitish round lesions sometimes in ladder-like streaks heavy infection: plaques coalescing, thickened intestinal wall	coagulation necrosis mucoid, bloody enteritis in lower intestine	thickened walls, mucoid, blood-tinged exudate, petechiae	light infection: rounded plaques of oocysts heavy infection: thickened walls coalescing plaques
MICROSCOPIC CHARACTERISTICS	MILLIMICRONS OOCYSTS REDRAWN FROM ORGINALS				
	LENGTH x WIDTH µm LENGTH = WIDTH =	AV = 18.3 x 14.6 17.7 - 20.2 13.7 - 16.3	24.6 x 18.8 20.7 - 30.3 18.1 - 24.2	30.5 x 20.7 21.5 - 42.5 16.5 - 29.8	15.6 x 13.4 11.1 - 19.9 10.5 - 16.2
	OOCYST SHAPE AND INDEX - LENGTH/WIDTH	ovoid 1.25	ovoid 1.31	ovoid 1.47	ellipsoid to broadly ovoid 1.16
	SCHIZONT, MAX IN MICRONS	10.3	30.0	9.4	17.3
LIFE HISTORY CHARACTERISTICS	PARASITE LOCATION IN TISSUE SECTIONS	epithelial	2nd generation schizonts subepithelial	gametocytes subepithelial	epithelial
	MINIMUM PREPATENT PERIOD-HR	97	120	121	93
	SPORULATION TIME MINIMUM (HR)	17	18	30	12

Compiled from various sources.

DIAGNOSTIC CHARACTERISTICS IN RED

E. mitis	*E. necatrix*	*E. praecox*	*E. tenella*
	large schizonts, no oocysts 		
no discrete lesions in intestine mucoid exudate	ballooning, white spots (schizonts), petechiae, mucoid blood - filled exudate	no lesions, mucoid exudate	onset: hemorrhage into lumen later: thickening, whitish mucosa, cores clotted blood
			
15.6 x 14.2 11.7 - 18.7 11.0 - 18.0	20.4 x 17.2 13.2 - 22.7 11.3 - 18.3	21.3 x 17.1 19.8 - 24.7 15.7 - 19.8	22.0 x 19.0 19.5 - 26.0 16.5 - 22.8
subspherical 1.09	oblong ovoid 1.19	ovoid 1.24	ovoid 1.16
15.1	65.9	20	54.0
epithelial	2nd generation schizonts subepithelial	epithelial	2nd generation schizonts subepithelial
93	138	83	115
15	18	12	18

Modified after Long and Reid (1982).

Source: (Conway and McKenzie, 2007).

Figure 2.Differential Characteristics for the 8 species of Chicken Eimeria

2.9. Treatment, control and Prevention

2.9.1. Treatment

The effective use of anticoccidial feed additives over the past 50 years has played a major role in the growth of the poultry industry and has allowed the increased availability of high quality, affordable poultry products to the consumer. These anticoccidials can be classified as chemicals which have specific modes of action against parasite metabolism (Table 3), such as amprolium, clopidol decoquinate, halofuginone, and polyether ionophores such as monensin lasalocid, salinomycin, narasin, and maduramycin, which act through general mechanisms of altering ion transport and disrupting osmotoic balance. These latter compounds are now the mainstay of coccidiosis control. A compendium of the most frequently used anticoccidials is available through Janssen Pharmaceutica. It is quite clear, however, (Chapman, 1994; Chapman, 1998), that some degree of resistance to all anticoccidial drugs, including ionophores, has developed. To minimize the effects of resistance, poultry producers rotate the use of various anticoccidials with successive flocks, combine chemical and ionophore treatments, or employ shuttle programs during a flock grow out. Application of these treatment programs depends on seasonal conditions and prevalence of various species of coccidia.

Table 3; Application of Some common drugs for treatment of coccidiosis in poultry

Name	Feed or water
Amprolium	Water
Chlortetracycline	Feed
Furazolidone	Feed
Nitrofurazone	Water
Oxytetracycline	Feed
Pyrimethamine + sulfaquinoxaline	Water
Sodium sulfachloropyrazine Monohydrate	Water
Sulfadimethoxine	Water
Sulfamethazine	Water
Sulfaquinoxaline	Feed

Source FAO ,1997

2.9.2. Control and Prevention

Coccidiosis is by far more easily prevented than treated; control depends mainly on drugs, although an effective vaccine is now available for breeders or layer replacements. Drugs have been very important in controlling coccidiosis but the emergency of coccidial drug resistance has affected the use of fullness of the drugs. The possibility that drugs may not always be relied up on to control coccidiosis has led to an interest in other means of control (Vegad, 2004). Apart from the use of drugs, control is now based on hygiene, vaccine and genetics. But genetics is a theoretical strategy not in practical use (Jordan *et al*., 2002). A program of preventive measures and immediate treatment in the event of an outbreak are very necessary if optimum overall performance is to be achieved (Whiteman and Bickford, 1989).

Prevention of avian coccidiosis is based on a combination of good management and the use of anticoccidial compounds in the feed or water. Litter should always be kept dry and special attention should be given to litter near water fonts or feeding troughs (Urquhart *et al*., 1996; Taylor *et al*., 2007). The prophylactic drugs used for prevention of coccidiosis are coccidiosistats. An effective coccidiostat should inhibit the schizogonic stage and allow immunity to develop. Prophylactic use is performed because most of the damage occurs before signs become apparent, and because drugs cannot completely stop an outbreak (Kahn, 2005).

The use of anticoccidial agents depends on the type of management concerned (Taylor *et al*., 2007). Layers and breeders that are maintained on floor must have protective immunity. Often, they are given a suboptimal dosage of anticoccidial drug during early growth expecting that immunity will continues to develop from repeated exposure to wild types of coccidia. Failures occur if the suppression is either inadequate or too great. Drugs vary in their ability to interfere with development of immunity. No immunity is necessary in chickens reared for meat production or in floor reared layers to be moved to cages for these exposure and infection should be minimized (Kahn, 2005).

Traditional control methods using chemicals (coccidiostats) are becoming less attractive due to increasing parasite resistance to chemicals, the need to minimise residues in the environment and in food, and the demands of alternative production systems (e.g. organic) (Chapman, 1997). Live vaccines offer an effective alternative to chemical control by producing species-specific protection. The benefits of using live vaccines include: long-term, economical protection against disease; ability to manage chemical resistance; and provision of an alternative to chemical control to minimise residue and withholding period problems for meat and eggs.

It is known that when chickens are infected with low number of *Eimeria* parasites, protective immunity is induced after two or three consecutive infections (Joyner and Norton, 1973; Long *et al*., 1986). Therefore, it would seem obvious that vaccines could offer excellent alternatives to drugs as a means of controlling coccidiosis. There by live

vaccines have been used mostly in breeder stocks and, to a lesser extent, in commercial broilers and replacement hens. This strategy is based on the well-documented protective immunity that develops in chickens after a primary coccidial infection.

Live vaccination, as indicated higher, is today less applied in broiler production. Two types of vaccines are discriminated, attenuated and virulent (Chapman *et al*., 2002). Attenuated vaccines lack a part of the life cycle (less asexual reproductive cycles) of the original strain they were derived from, and as a consequence have a lower reproductive and pathogenic potential. This is a major advantage towards performance of virulent coccidial vaccines, but because of the lower reproductive potential of attenuated vaccines, production costs are significantly higher. All commercially available coccidiosis vaccines are based on this principle. Depending on the characteristics of the vaccine strains used, these vaccines may be divided into three groups.

- Live, virulent strain based vaccine
- Live, attenuated strain based vaccines
- Live, ionophore tolerant strain based vaccine

2.10. Economic importance of poultry coccidiosis

Coccidiosis is one of diseases of poultry that play inhibitory role in the growth of this industry. It is a disease complex of poultry caused by different species of parasite of *Eimeria.* It inflicts the birds in both clinical and sub-clinical forms. The clinical form of the disease manifests through prominent signs of mortality, morbidity, diarrhoea or bloody faeces, and sub-clinical coccidiosis manifests mainly by poor weight gain and reduced efficiency of feed conversion and gives rise to highest proportion of the total economic losses (Williams, 1999).

Coccidiosis is recognized as the parasitic disease that has the greatest economic impact on poultry production. The annual worldwide cost is estimated at about $800 million (Williams**,** 1998), and that for the American broiler industry about $450 million. These estimates include the costs of prophylactic in-feed medication for broilers and broiler-

breeders, alternative treatments (e.g., with amprolium) if the medications fail, and losses due to mortality, morbidity, and poor feed conversions of birds that survive outbreaks.

The impact of disease on animal agriculture is typically assessed in quantitative terms. In poultry industry, these terms include for example lost revenues; costs of vaccination/prevention, eradication, decontamination and restocking. These have been referred to as negative inputs (Thrusfield, 1995). In Ethiopia, diseases are among the major factors that hinder poultry development (Alamargot, 1987). In this developing country, poultry mortalities due to diseases are estimated in between 20% to 50% but they can rise as high as 80% during epidemics (Alamargot, 1987; Alemu, 1995). Poultry coccidiosis is one of these diseases causing significant poultry losses in Ethiopia.

In Ethiopia coccidiosis was identified as a cause of direct and indirect losses in all farms. Losses occurred in the form of mortalities, coccidiostats cost, reduced weight gains, reduced market value of affected birds, culling, delayed off take and reduce egg production. Average losses due to mortalities, culling and coccidiostats costs were estimated at Ethiopian Birr 898.80 and 5301.80 per farm or 0.55 and 0.53 Ethiopian Birr per chicken in small scale and large scale poultry farms, respectively. This contributed to an average of 11.86% and 8.40% loss in enterprise profit per farm in small and large scale farms, respectively. Proportional mortality rates due to coccidiosis were 14.5% and 13.3% in small scale and large scale poultry farms, receptively (Safari *et al*., 2004).

2.11. Current status of poultry coccidiosis in Ethiopia

Poultry coccidiosis is endemic in Ethiopia, causing great economic losses particularly in young birds, in all production systems (FAO/ILRI, 1995). For instance prevalence rate of 50.8% and 11% in deep litter intensive system and backyard extensive production system, respectively was reported from Debrezeit and its surrounding (Fessessework, 1990). Moreover study on the occurrence of coccidiosis and distribution of *Eimeria* species in dead chickens of 1-60 days of age, at Kombolcha Poultry Multiplication and Research Centre (KPMRC), Ethiopia indicated that out of 965 dead birds, 370 (38.34%)

were found to have clinical coccidiosis. The *Eimeria* species identified in those studies were *E. brunetti*, *E. tenella*, *E. acervulina* and *E. necatrix* with prevalence of 45.3%, 40.8%, 9.7% and 4.1%, respectively (Lobago *et al*., 2005).

Poultry occidiosis, caused by for example *Eimeria acervulina, Eimeria necatrix, Eimeria maxima* and *Eimeria tenella*, is endemic in all parts of the country and affects mainly young growing birds (Safari *et al*., 2004). The species of coccidia identified in Ethiopia are *E. tenella*, *E. necatrix*, *E. maxima* and *E. acervulina*, E. *mivati* and E. *brunetti* (Guale, 1990; Ashenafi, 2000; Methusela, 2001; Lobago *et al*., 2005).

Studies conducted in Arsi zone (Tiyo dstrict) revealed prevalence rates of 22.58% and 12.25% for clinical coccidiosis in rhode Island red(RIR) and local strain chickens respectively (Gari *et al,* 2008). Additional investigation done in central Ethiopia revealed that *Eimeria acervulina* was the most prevalent coccidial species (Hagos, 2000; Safari, 2001; Lobago,*et al*, 2003), whereas study conducted in Kombolcha disclosed that *Eimeria burneti* was the most prevalent coccidian species . This variation may be because of the possibility of drug resistance or/and differences in virulence of Eimeria species under different management systems (Table 4).

The possible risk factors associated with the outbreak of coccidiosis in Ethiopia were reported as absence of proper disposal of litters, wetting of litters from leaking pipes, absence of all- in all- out system, the presence of stressors (such as change in diets and concurrent infections) and extensive use of coccidiostats (Mersha *et al.*, 2009). Moreover, (Gari *et al*., 2008) have reported that the potential risk factors for the occurrence of coccidiosis in free-ranging local chickens were non-selective picking behavior during feeding, age group, high moisture conditions whereas in Rhode Island Red (RIR) breeds that were kept under intensive deep litter system, the potential risk factors observed from farm assessment through questionnaire were age groups, production systems, flock size, moisture level in the poultry house and level of biosecurity.

Table 4: Prevalence of coccidiosis in different areas of Ethiopia.

Site of study	Prevalence	Reference
Debre zeit	71.7%	Dinka and Yakob, 2012
Komblcha	25.24%	Abdi *et al*,2012
Adiss Ababa	23.1%	Alemayehu *et al*, 2012
Ambo	20.57%	Oljira *et al*., 2012
Arsi Tiyo District	64.4%	Gari *et al*, 2008
Central Ethiopia	25.8%	Ashenafi *et al*, 2004

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in and around Ambo town, West Shewa zone of Oromia regional State, Ethiopia starting from October, 2013 to February, 2014. Ambo town is the administrative center of the zone and located at a latitude and longitude of 8°59′N 37°51′E8.983°N 37.85°E and an elevation of 2101 meters above sea level (asl) and 114 Km west of Addis Ababa and the Ambo woreda has 34 administrative kebeles (ATMA, 2010) map of the study area are indicated on (Fig, 3).

The agroecology of the study area is 23% highland, 60% midland, and 17% lowland. It has an annual rainfall and temperature ranging from 800 – 1000 mm and 20^{o} – 29^{o}c respectively. The rainfall is bi-modal with the short rainy season from February to May and long rainy season from June to September (AARDB, 2006). The livestock population of the district includes 145371 cattle, 50152 sheep, 27026 goats, 105794 chickens, 9088 horses, 2914 donkeys and 256 mules.

Traditional chicken production is still the most dominant even though there are some initiations for introduction of exotic chicken in and around urban areas of the zone. Both local and cross breeds chicken are raised in the area. Local breeds are reared under extensive farming system where as cross breeds of egg laying type's mostly in semi-intensive ones and very few are under extensive farming system (AARDB, 2006).

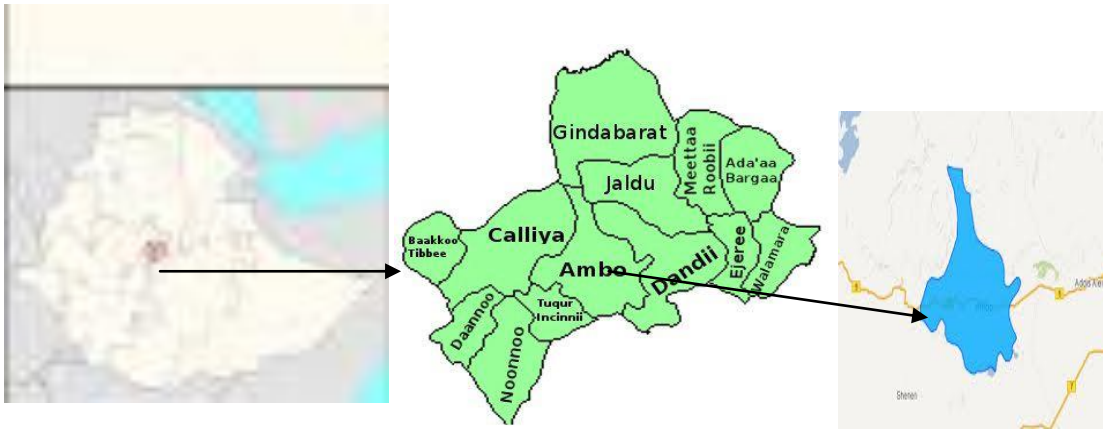


Figure 3:- Map of study area

3.2. Study population

The study animals were local breed and Rhode Island Red breed of chickens which were bought directly from the farmers randomly from markets and from selected farms. A total of 390 chickens from both sexes were included proportionally as male and female and short interview of owners about the pedigree of their chickens were made to distinguish local breeds from exotic breed chicken. Age of the chickens was determined by observing color of the shank and growth of the spur and most of the chickens bought were approximately in the range of growers or young (4-12 weeks of age) and adult (greater than 12 weeks of age). Then the chickens were transported to Ambo University, department of Veterinary Laboratory Technology laboratory for ante-mortem, postmortem and parasitological examination.

3.3. Study design and sample size determination

A cross sectional study design was conducted from October 2013 to February 2014 to determine the prevalence of coccidiosis in local strain and RIR chicken, to identify the

prevalent species of Eimeria in the study area and to determine the risk factors of coccidiosis (management, sex, breed and age).

Sample size was determined based on the assumption of possible or expected prevalence rate of the disease recorded in the study area which was 20.57% (Olijira *et a.l*, 2012). The formula applied to calculate sample size was the formula for simple random sampling method since it is used as study method and the study has considered 95% confidence level and 5% absolute precision (Thrusfield, 2005).

$$n = Z^2 * \frac{(p_{exp} * q)}{d^2}$$

n= the required sample size

Pexp= Expected prevalence

q = (1- Pexp)

d = Desired absolute precision (5%)

Where Z (a multiplier for 95% confidence interval based on the normal distribution) =1.96, p=20.57%% and d=5%. Therefore, a minimum required sample size was 251. However, 390 chickens were sampled to increase precision.

3.4. Study methodology

3.4.1 *Ante-mortem examination*

Simple questionnaire was conducted by using interview of owners at the time of purchase of chicken to assess risk factors like breed, age, management systems, history of coccidiosis in their poultry, other problems of the poultry, etc. Observational assessment was made at the same time to assess managemental practices in semi-intensive poultry farms. Each purchased chickens were kept overnight in the laboratory for at least 18 hours in separate cartoon and a clean plastic wrap. Fecal samples were collected from each bird separately and ante-mortem condition of individual chicken was checked.

3.4.2. *Postmortem examination*

After 18-24 hours stay of chicken in the laboratory for ante mortem examination the chickens were killed by neck dislocation following procedures of Zander and Mallinson (1978). The intestinal tract was opened with scissor, extending from the duodenum to the rectum including both cecal pouches. The gastrointestinal tract had grossly examined carefully described by Lobago *et al.* (2005) and Gari *et al.* (2008). The intestinal portions were divided into 4 sections, the upper part (duodenum and jejunum), the middle part (ileum), lower part (distal ileum and rectum) and cecal pouches. The intestinal walls of different sites were examined for thickening, petechiae, coagulative necrosis, reddening, whitish spots, cecal cores, or bleeding. The observed gross pathological lesions were recorded.

3.4.3. *Parasitological examination*

The faeces and intestinal scraping, which was taken after postmortem, of each bird were collected in separate petri-dish and the intestinal pooling sample of each individual bird was blended by mortar and pistol and then floatation technique using sodium chloride solution were applied to harvest oocyst (Bowman, 2003) (Annex 1). Calibration of the objective lens was done based on the procedures described by Conway and McKenzie, (2007).Then the average length and width was measured using ocular micrometer from at least 3-5 oocysts to determine the size. Positive samples were further examined for species identification.

Sporulation time of the oocysts was identified by culturing for different time length to identify the *Eimeria* species. To determine the sporulation time of the oocysts, the floatation fluid was removed by washing the oocysts with tape water and centrifuged 3-4 times. The floatation solution-free oocyst suspension was preserved in 2.5% potassium dichromate solution. Thin layer of oocyst suspension in 2.5% potassium dichromate was added into Petri dishes and allowed to sporulate at room temperature, which the day temperature ranges in average 18-22^{0}C (Conway and McKenzie, 2007). The procedure

for oocyst sporulation is described in (Annex 2). The suspension was examined by hemocytometer chamber every 3 hours during the working hours to determine the sporulation time and the sporulation time was considered when 90% of the oocysts were sporulated. After the sporulation time is determined the oocysts was preserved in 2.5% potassium dichromate and stored at 4^0C. Oocyst color and size is mostly helpful characteristics for identification of *E. maxima* that is brown red color and significantly large in size. The length and width of the individual oocyst were measured using a calibrated ocular microscope (Annex 4, Fig. A). The average sporulation time and range of size of each species and their identification characteristics are shown in (Table 2). Most coccidial species were restricted to specific predilection sites in the intestine.

3.4.4. *Histopathological examination*

The observed gross pathological lesions was recorded and sampled for histopathological examination. Tissue samples of intestines, about 3-4 cm length, were sampled and fixed in 10% neutral buffered formalin and submitted to National Animal Health Diagnostic and Investigation center (NAHDIC), Ethiopia. The tissue samples were dehydrated in ascending order of alcohol concentration, cleared in xylene, embedded in paraffin wax, sectioned at 4μm thickness and stained with haematoxylin-eosin according to procedures described by Talukder. S, (2005) (Annex 3). The stained samples were examined by light microscope for histological changes by using 10 times and 40 times magnification power.

3.5. Identification of species of *Eimeria*

Species of *Eimeria* were identified by combination of microscopic features of oocyst morphology (shape, size, sporulation time and color of the oocysts), the predilection site of *Eimeria* in the gut, the nature of gross lesions induced and histopathological finding as described by Conway and Mckenzie (2007).

3.6. Data analysis and management

The data collected were coded and entered into Microsoft Excel and descriptive statistics was utilized to summarize the data using Software program for Social Science (SPSS) statistical software version 20. The point prevalence was calculated for all data by dividing positive samples by total number of examined samples and multiplied by hundred. Chi-square test (Fisher's exact for data with a frequency of less than five in cell) was used to assess if there were a statistically significant difference in poultry coccidiosis infection between sex, breed, age, and management groups. For this analysis p-value less than 0.05 was considered as significant where as p-value greater than 0.05 considered as non significant.

4. RESULTS

4.1. Prevalence of coccidiosis

Out of 390 examined chickens, 73(18.7%) were positive. The prevalence of coccidiosis by age group of young and adult poultry was recorded as 20.78% and 16.98%, respectively and there is no statistical significance difference in prevalence of the disease between age groups (Table 5) (p> 0.05 The result that was obtained between local or indigenous and rode island red (RIR) breed are shown in (Table 5) and the result revealed absence of statistical significance difference (p> 0.05).

Table 5: P**revalence of coccidiosis by age, sex, breed and management**

Variables		№ of poultry examined	№ of positive(%)	χ^2 value	p-value
Age	Young	178	37(20.78)	0.921	0.337
	Adult	212	36(16.98)		
Sex	Male	129	35(23.49)	3.609	0.057
	Female	241	38(15.76)		
Breed	Local	265	55(20.75)	2.245	0.133
	RIR	125	18(14.4)		
Management system	Extensive	303	58(19.14)	0.16	0.689
	Semi-intensive	87	15(17.24)		
Total		390	73(18.7)		

P > 0.05:

There was no significant difference observed in the prevalence of coccidiosis among the different variables

4.2. Gross lesion finding

Post mortem examination is the best method for diagnosis and species identification. The site of their occurrence and characterstics lesions produced by specific *Eimeria* species were used for identification of the species (Table 2). *E. tenella* is one of the most prevalent species that induced haemorrhage, clotted blood and cecal cores (Annex 4, Fig. B) in the cecum. *E. necatrix* and *E. maxima* usually shared similar intestinal lesions. Lesions in *E. necatrix* were more sever with bleeding (Annex 4, Fig.C) and whitish plaques seen in the middle intestine on both sides from yolk sac diverticulum's. Lesions induced by *E. acervulina* usually seen in the duodenal loop and characterized by mucoid exudates in intestinal content and white spots usually evident from the serosal side.
E. burnette was parasitizes the lower intestine, large intestine (between the ceca) and rectum. *E. burneti* induced petechial haemorrhage was recognized on the serosal than the mucosal surface, that appeared in small areas.

4.3. Histopathological examination

Histological sections prepared from classical lesions of *E. tenella* and *E. burnette* were examined. Pathological lesions observed in the cecum showed sever tissue damage and plenty of schizonts (Annex 4, Fig. D). The cryp cells were highly invaded with the developmental stages of *E. tenella* schozonts and gametocytes that their morphology is almost disappeared. The mature and immature schizonts have occupied the large proportion of the cryp cells (Annex 4, Fig. E). The lesion in the lower intestine due to *E .burnette* also consisted cryptal and absorptive epithelial cell destruction. Large number of gametocytes had observed in stained section of the epithelial tissues and inflammatory cells infiltrated into the lamina porpria were also observed (Annex 4, Fig C and F). Hetrophil cells predominantly infiltrates into the site when necrosis was intensive (annex 4, Fig. F).

4.4. Species identification

In the current study; five *Emerian* species, namely *E. tenella, E. necatricx, E. burnette, E. maxima* and *E. acervulina* were identified. All these species were identified as a sole infective agent and mixed infective agents. Mixed infection cases were recorded due to *E. tenella* together with any of the four species (*E. necatrix. E. burnetti*, *E. maxima* and *E. acervulina*) infection with *E. burnette* and *E. necatrix* as shown in (Table 6).

The distribution and frequency of *Eimeria* species at different risk factors as either single or mixed infections were not significantly different ($p > 0.05$) as indicated in (Table 7). The *Eimeria* species identified either single or mixed infections at different determinants *E. tenella* and *E. necatrix* occurred most frequently with prevalence of 62.2% and 18.29%, respectively, whereas the rest three species identified, *E. burnette, E. maxima* and *E. acervulina* were low in prevalence and accounts prevalence rates of 10.98%, 6.09 and 2.43%, respectively (Table 7). The result showed overall prevalence of 87.67% (64/73) and 12.32% (9/73) for single and mixed infection, respectively.

Table 6: Distribution of *Eimeria* species as single and mixed infection

Species of *Eimeria* as single and mixed	№ of positive (frequency)	Proportion
E. tenella	44	60.27%
E. necatrix	11	15.06%
E. burneti	7	9.6%
E. maxima	1	1.4%
E. acervulina	1	1.4%
E. tenella + E. burnette	1	1.4%
E. tenella + E. necatrix	2	2.7%
E. tenella + E. maxima	4	5.5%
E. tenalla + E. acervulina	1	1.4%
E. necatrix + E. burnette	1	1.4%
Total	73	100%

Table 7: Frequency of Eimeria species identification from examined chicken in different category of variables (n=73)

Variables		Frequency of Eimeria species (%)					
		E. tenella	*E.necatrix*	*E.burnette*	*E.maxima*	*E. acervulina*	Total (%)
Age	Young	22(43.14)	10(66.67)	5(55.56)	4(80)	2(100)	43(52.44)
	Adult	29(56.86)	5(33.33)	4(44.44)	1(20)	0	39(47.56)
	χ^2 value	0.148	2.78	0.365	2.41	2.394	
	P value	0.700	0.95	0.737	0.183	0.208	
Sex	Male	24(47.1)	7(46.67)	4(44.44)	3(60)	1(50)	39(47.56)
	Female	27(52.9)	8(53.33)	5(55.56)	2(40)	1(50)	43(52.44)
	χ^2 value	1.948	0.473	0.152	1.019	0.118	
	P value	0.163	0.492	0.737	0.375	1.000	
Breed	Local	40(78.43)	9(60)	6(66.67)	5(100)	1(50)	61(74.39)
	RIR	11(21.57)	6(40)	3(33.33)	0	1(50)	21(25.61)
	χ^2 value	2.960	0.453	0.007	2.389	0.297	
	P value	0.085	0.501	1.000	0.181	0.537	
Management system	Extensive	41(80.4)	11(73.33)	7(77.71)	5(100)	1(50)	65(79.27)
	Semi-intensive	10(19.6)	4(26.67)	2(22.22)	0	1(50)	17(20.73)
	χ^2 value	0.247	0.171	0.000	1.454	0.890	
	P value	0.619	0.751	1.000	0.591	0.397	
	Total	51(62.2)	15(18.29)	9(10.98)	5(6.09)	2(2.43)	82(100)

5. DISCUSSION

The result of the present study illustrate that poultry coccidiosis is endemic in and around Ambo town, Ethiopia with an overall prevalence of 18.7% (73/390) coccidiosis was registered in this study. The present result agreed with the finding in central Ethiopia (Ashenafi *et al*, 2004), Addis Ababa (Alemayehu *et al*, 2012) and in Kombolcha(Abadi *et al* , 2012)with prevalence rate of 25.8%, 23.1% and 22.3%, respectively. Moreover, this result was in agreement with the finding in Ambo (Diriba *et al*, 2012) and Arsi Tiyo (Gari *et al* , 2008) who reported a prevalence of 20.57% and 22.58%, respectively. This relative prevalence of the infection in the sampled chickens might be due to management and the period of the study that may concede the same season. However, the present result in the prevalence of coccidiosis is much lower than the findings of Dinka and Yacob (71.1%), Gari (70.95%) and Alemargot (80%). This high reduction of prevalence of coccidiosis observed in the current study might be ascribed mainly to the application of preventive measures which basically sanitary measures and use of anti coccidial drugs that were given at early stages of age. Other point may be due to breed difference, improvement of management system and bio security measures when compared to the setup in the previous study.

In this study, the prevalence of coccidiosis was 20.78% in young chickens while in adult 16.98%. It was observed that there was no statistically significant difference ($p>0.05$) in the prevalence of coccidiosis among the two different age groups examined. However, slight variation was observed between the age categories. This may be due to management system and breed factors. The amount of oocyst discharged from infected chicken depends on the dose of oocysts ingested and the immunological status acquired from previous exposure (Willians, 2005). Thus, the opportunity of the chickens to pick-up large numbers of sporulated oocyst might be equal in both young and adult chicken. The current prevalence is lower compared to the result of other survey in Nigeria (Muazu *et al.,* 2008) and in Pakistan (Bachaya *et al*, 2013) reported 52.9% and 36.6%; 60.16% and 37% in young and adult, respectively.

Although the current survey was conducted in dry season when coccidiosis is reported to decrease due to decreased floor wetness, but due to poor management in local chicken in and around Ambo, the prevalence of coccidiosis is slightly higher in local breed than RIR breed (Table 5). The prevalence of coccidial infections in local breed chickens in the present study was lower than in previous finding (Gari *et al*, 2008; Ashenafi *et al*, 2004). The climatic condition, agro-ecological setup and management system on the subject might be attributed to the variation. The relatively dry climate in the mid and low altitudes of the study area may be more favorable for the decrement of coccidiosis. In another way, this finding is in line with the finding of Ahmed *et al.* (2012) in Egypt and Tehetena (2010) who recorded 21.24% and 23.1%, respectively. The prevalence of coccidial infections in RIR breed was 14.4% in current study. This result is not in agreement with the finding in large and small scale deep litter rearing system (Mathusela *et al.,* 2002 and Gari *et al.,* 2008). In the current study occurrence of coccidiosis was statistically not significant between the sex groups. This indicates there is no significant natural resistance variation in relation to the sex (Pinard *et al,* 1998).

Among the two managemental practices, poultry reared under semi-intensive farm system showed 17.24% infection while as the extensive (un-organized) farming systems harbored 19.14% infection with *Eimeria* species. The prevalence was statistically not significant ($p>0.05$). However, in the present study, percentage prevalence of infection in extensive farming system has slight higher than from intensive farming system. The difference observed may be due to poor management system, malnutrition and non used anticiccidial drug in back yard chickens. The warmth and moisture environment favors greater transmission and contamination of oocysts. The present finding is in line with the finding in India by (Sourabh *et al,* 2013) who reported that higher prevalence in extensive than in intensive management systems, 53.6% and 25.55%, respectively.

Most of the chicken sampled had unapparent and low grade coccidian infections. This may call for concern, since the economic implication of coccidiosis is largely associated with the subclinical form of the disease as it has been reported to have negative effect on the performance of infected poultry (Haug *et al.*, 2008). Impaired feed conversion is

among the major comprise some 70% of the cost of producing commercial chickens; the economic impact of subclinical infection is therefore considerable. The biological characteristics of chickens are well known and variables, and can be used in the identification of species (McDougald, 2003).

In the present study, five important species of *Eimeria* were identified, namely *E. tenella, E. necatrix, E. burnette, E. maxima* and *E. acervulina* with the prevalence rate of 62.2%, 18.29%, 10.98%, 6.09% and 2.43%, respectively. Some species are easily identified on the basis of oocyst size (*E. maxima*), where as others produce unmistakable lesions and site of lesions that is occurred (*E. tenella, E. necatrix, E. burnette* and *E. acervulina).*
These results are in agreement with the previous reports from Ethiopia (Mathusela *et al*, 2002; Ashenafi *et al.,* 2004; Abdi *et al.*, 2012; and Dinka and Yacob, 2012) reports four species of *Eimeria* except *E. maxima.* However, reports in Nigeria by Muazu *et al*, (2008); in Iran Mohamed *et al*,(2011) and in Pakistan Bachaya *et al*,(2013) were also reported four species of *Eimeria* except *E. burnetti* , suggesting that those species of *Eimeria* are wide spread in many countries. The prevalence of infection in studied chickens in the current study indicates the maintenance of oocysts in the farm and external environment, improper cleaning and disinfection methods in the chicken house. In the present study *E. tenella* (62.2%) was the predominant species, followed by *E. necatrix* (18.29%). This finding is in agreement with the finding of (Dinka and Yacob, 2012; Gari *et al*, 2008). However, previous research conducted in Ethiopia by (Ashenafi *et al*, 2004; Safari, 2001) and in Iran (Ahmed Nematolhi *et al* 2004) revealed that *E. acervulina* was the predominant species of *Eimeria*. On the other hand (Lobago *et al*, 2005) reports in Ethiopia that *E. burnetti* was the most prevalent species.The probable reasons for this discrepancy could be the differences in virulence of the *Eimeria* species at different management system and /or due to the prophylaxis use of anti coccidial drugs in feed and water or may be due to breed differences. It is likely that resistance has developed to more recent anticoccidial drugs (Chapman, 2005) and very few drugs are equally efficacious against all *Eimeria* species (McDougald, 2003). The occurrence and incidence of coccidiosis is also, to a great extent affected by the type of chick reared and breed sensitivities to infection (Tyalor *et al.,* 2007). Many coccidiostatics drugs have

been directed against *E. tenella*, with the result that other species are increasingly incriminated as a cause of poultry coccidiosis (Urquhart *et al.*, 1996).

The gross and histopathological changes observed for each species were quite similar to what was previously described in other studies (McDouglad, 2003; Williams, 2005; Haug *et al*, 2008 and Amer *et al*, 2010). The observed lesions caused by *E. maxima* and *E. necatrix* infections might be occasionally misdiagnosed as coccidiosis, particularly when concomitant with coccidiosis (Catchpole, 2000) and necrotic enteritis (Wages and Opengart, 2003). Nevertheless, the finding of this study was confirmed by mucosal scraping examination and histopathological and found that it was due to coccidial parasites. Mixed infections were frequently encountered, which account for nine mixed infection cases. The larger proportion of mixed infection consisted of *E. tenella* which may be due to the wide spread distribution of this species in the study area.

6. CONCLUSION AND RECOMMENDATIONS

In conclusion, despite the reduction in the prevalence of coccidiosis in the present study, coccidiosis is a major burden to poultry producers and veterinary health professionals. The occurrence of coccidiosis in different age categories was not statistically significant differences. However, prevalence of coccidiosis was slightly higher in young age group than adult birds. This might be the adult birds develop immunity to the trickle infections acquired from the environment and maintain the state of balance to the infection.

Prevalence of coccidiosis in local breed is slightly higher than the Rhode Island Red breed. This result indicated may be due management practice of RIR breed to cope up the effect of the disease. In general terms, differences in resistance to coccidiosis in relation to sex and management were not significant. Slightly higher prevalence of the infection in studied native chicken in the current study indicates the maintenance of oocyst in the environment, improper cleaning and disinfections methods in the local chicken house and indiscriminate scavenging behavior. To conclude that coccidiosis in local chickens in the present study is slightly greater than the RIR breed.

The coccidia species identified were *E. tenella, E. necatrix*, *E. burnette, E. maxima* and *E. acervulina* while *E. tenella and E. necatrix* were the predominant species. The infection rate detected in this study may suggest for the presence of favorable condition for biology and transmission of the pathogen. The distribution of isolated species of Eimeria was not significantly different between different age groups. The effect of coccidiosis on the production ability of chicken and its economic importance should be further studied.

Therefore, the following recommendations are forwarded:
Efforts toward educating the local chicken farmers especially in villages to control coccidiosis through good management practices, and the proper use of anticiccidial drugs should be considered.

It is necessary to maintain good hygiene and sanitation in the farm as disinfectants are not effective against coccidia, the following point should be considered to maintain good hygiene

- ✓ Put waterers and feeders at height level with backs of the birds
- ✓ Clean the pens and remove infected dropping
- ✓ Keep older birds away from young (chick), since old birds are carriers.
- ✓ Avoid moisture and humidity in litters
- ✓ Keep the litter dry by frequent turning of the litter to reduce the sporulation of the oocyst.
- ✓ Avoid over-crowding in the house.

To control this economically important parasitic disease of poultry, further studies need to be undertaken to devise sustainable and cost-effective prevention and control methods.

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8. ANNEXES

Annex 1: Method of Flotation (Bowman, 2003)

1. 3grams of the intestinal pooling are suspended in 20 to 50 ml of water and mixed thoroughly
2. The mixture is then strained through a metallic sieve in to centrifuge test tube
3. The mixture is centrifuged at 2000 r.p.m for 2 minutes
3, then supernatant fluid is discarded.
4, flotation fluid (sugar solution) is added in to the test tube until slight convex meniscus formed at the top
5, then the cover slip is placed on the top of the test tube, making sure no air bubbles are present; then allow to stand for5-10 minutes
6. Then the cover slip is removed carefully and place on the microscope slide and
7. Then the slide examined under the microscope

Annex 2: Procedure to harvest oocysts from fecal samples and preservation (Conway and McKenzie, 2007)

1. The fecal material will be homogenized by blender until the fecal materials can be easily filtered through tea strainer sieve.
2. The solid materials will be discarded
3. The filtrate suspension was filled in to test tubes and centrifuged at 2000rpm for 5 minutes.
4. The supernatant fluid was discarded and the solid pellet was resuspended by saturated salt solution and the pellet was thoroughly mixed with the salt solution.
The test tube was filled by the salt solution up to 1cm below the brim.
5. The test tube was moderately centrifuged at 1500rpm for 5 minutes
6. The oocysts float on the top of the supernatant fluid.
7. A clean Pasteur pipette is used to take the oocysts from the top layer and put on the microscope slide, examined under 40x objective and 10x ocular micrometer eyepiece

using the calibrated microscope. The average length and width, shape and color of the oocysts were measured. Calibration of the objective lens was done based on the guideline described by Conway and McKenzie, (1991). To determine the sporulation time of the oocysts the salt solution was removed by washing the oocysts with tape water, centrifuge 3-4 times. The salt-free oocyst suspension was stored in 2.5% Potassium dichromate solution. The technique to initiate the sporulation of oocyst was described by Conway and McKenzie, (1991).

Thin layer of oocyst suspension in potassium dichromate 2.5% will be added into Petri dish and maintained at room temperature.

- The Petri dishwill put on electric shaker and adjusted to move in a gentle motion to aerate the suspension.
- The suspension will be examined by hemocytometer chamber every 3 hours during the working hours to determine the sporulation time and the sporulation time will be considered when 90% of the oocysts should be sporulated.

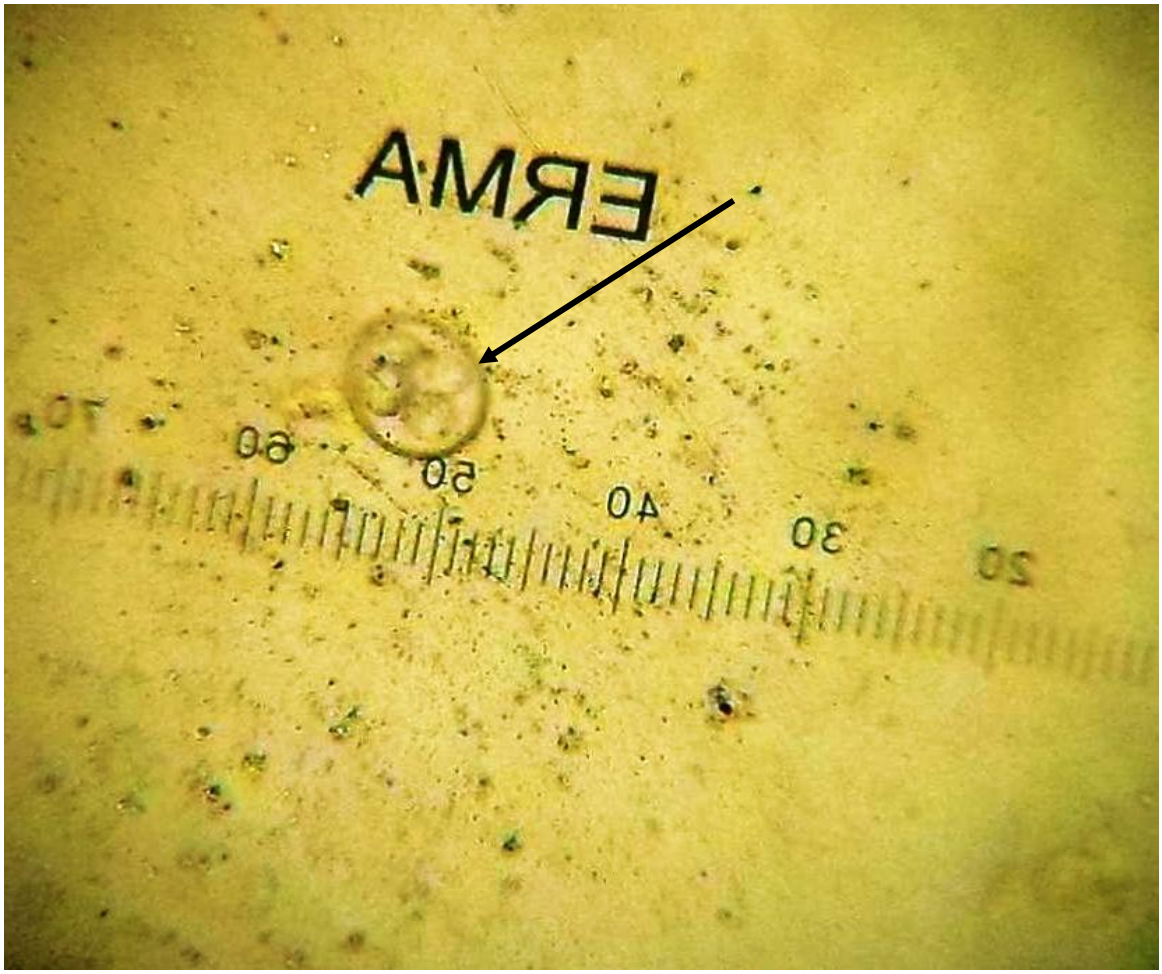
Annex 3: Hematoxyline eosine stain procedure (Talukder.S, 2007)

Staining Procedure:

1. Deparaffinize slides in 3 changes of xylene for 3 minutes each.
2. Hydrate slides in 100% alcohol and 95% alcohol, 2 changes for 3 minutes each, and rinse in distilled water until ripples disappear from slides.
3. Place in Hematoxylin for 8 - 15 minutes.
4. Rinse in tap water until water runs clear.
5. Decolorize in 1% acid alcohol, 3 - 6 quick dips. Check differentiation microscopically: Nuclei should be distinct; Cytoplasm should be uncolored.
6. Rinse in tap water until ripples disappear from slides.
7. Dip in Bluing Agent, 3 - 5 long dips.
8. Wash in luke-warm tap water for 5 minutes (37-40°C.)
9. Stain in Eosin for 30 seconds - 2 minutes.
10. Dehydrate in 95% alcohol and 100% alcohol, 3 changes each for 2 minutes.
11. Clear in 3 changes of xylene for 2 minutes each.
12. Mount coverglass

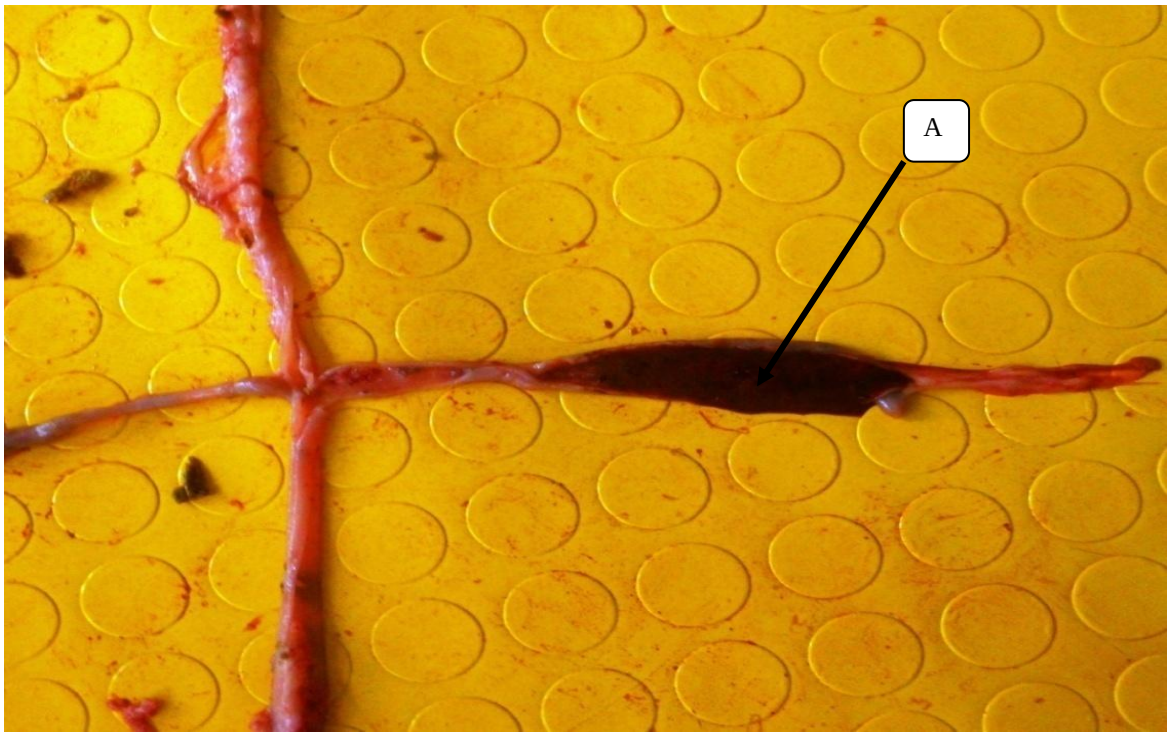
Annex 4: List of Figures

A. Arrow shows Sporulated *E. tenella* oocyst (400x).



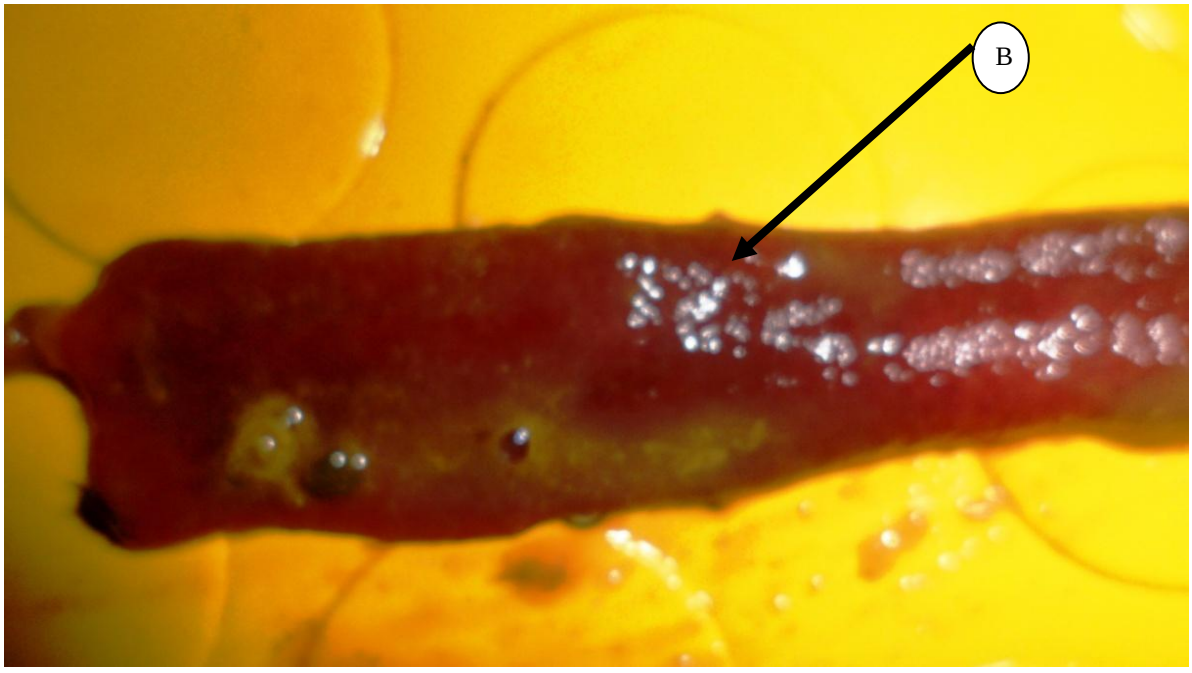
B. Lesion in the cecum caused by *E. tenella*

Arrow A show opened cecum filled with clotted blood and mucosal ulceration

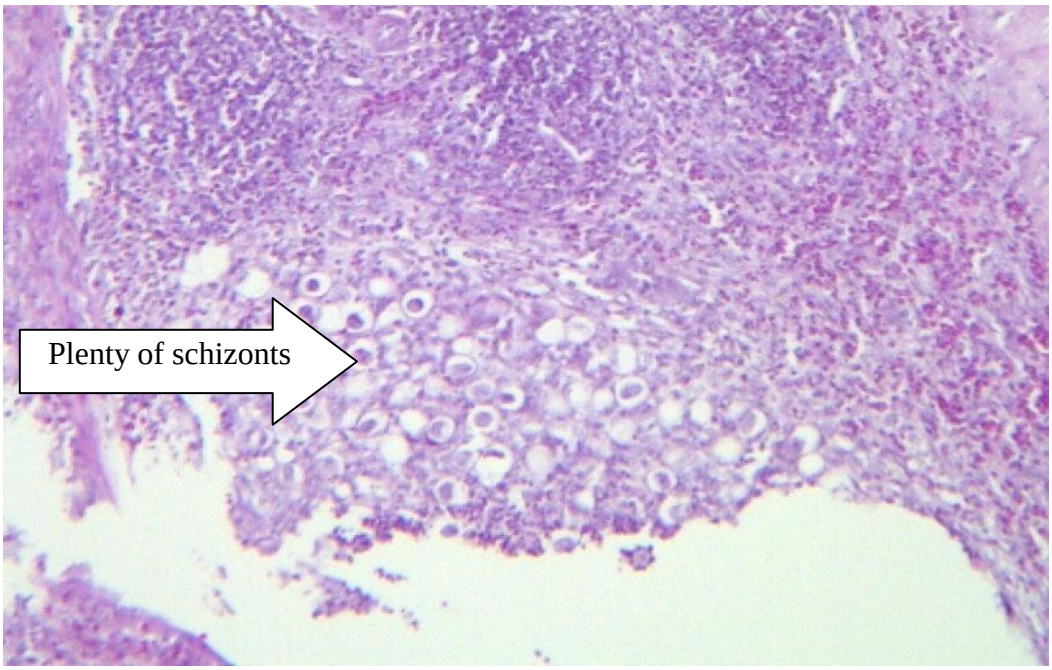


C. Lesion in the middle intestine caused by *E. necatrix*

Arrow B indicate opened intestine, thickening of the intestinal wall, with hemorrhage and bleeding and necrotic lesion in the mucosa.

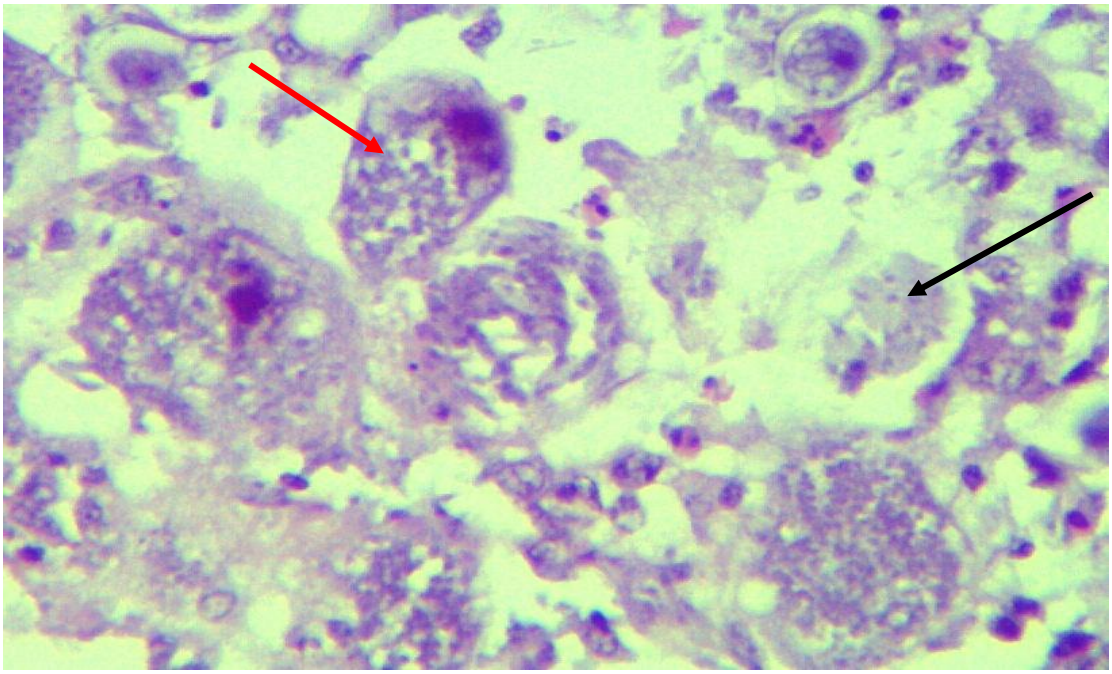


D. cecum section infected with *E. tenella* (100x).



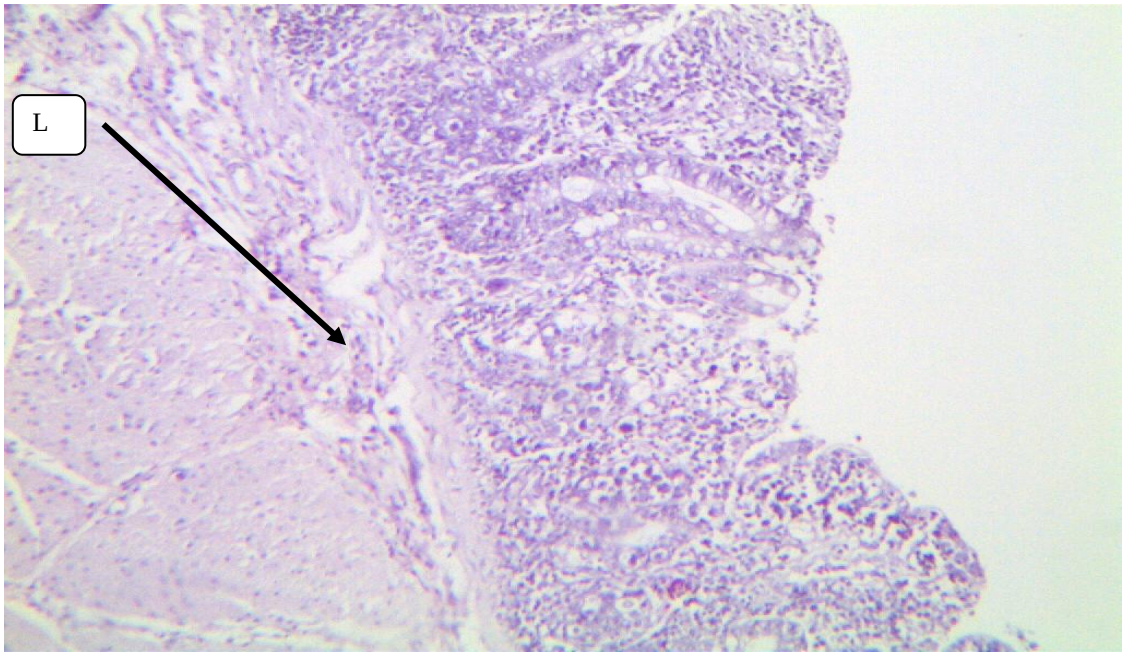
E. ecum section infected with *E. tenella* (400x).

Red arrow indicates mature schizointes and black arrow indicate immature schizonts of *E. tenella*.

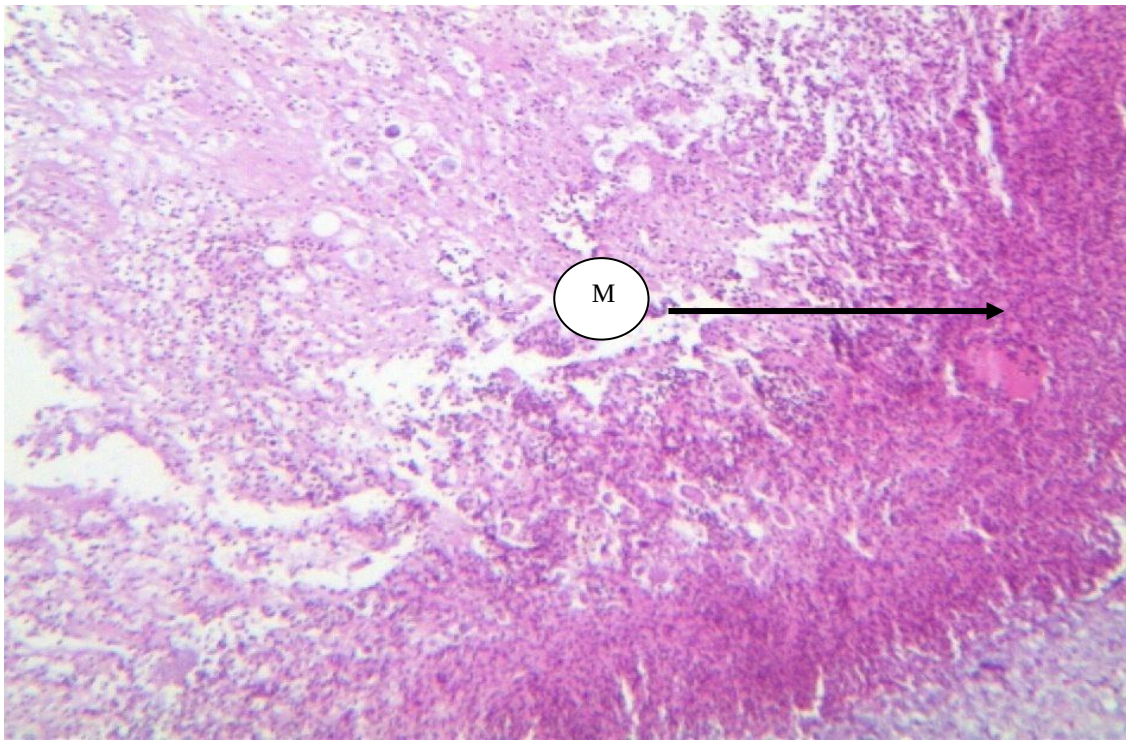


F. Large intestine section infected with *E. burnetti* (100x)

Arrow L shows high inflammatory cells influxes in to the sub mucosal layer



G. Arrows M indicate the presence of sever hemorrhage caused by *E. necatrix*.



Annex 5: *Eimeria* species identification format sheet

Sample code	OOCYST				lesion		Histopathological finding	Eimeria species
	Shape	Size	Color	Sporulation time	Location	score		

Annex 6: Data collection format sheet

Chicken number	Age	Sex	Breed	Origin	Management system	Result