ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

STUDIES ON POULTRY COCCIDIOSIS IN TIYO WEREDA, ARSI ZONE, OROMIA REGIONAL STATE

BY
GETACHEW GARI JIMOLU

JUNE, 2004
DEBRE ZEIT, ETHIOPIA

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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 Medicine

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GETACHEW GARI JIMOLU

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Declaration

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

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This thesis has been submitted for examination with our approval as University advisors.

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DEDICATION

This thesis paper is dedicated to my beloved wife Aynalem Gezehagn, my daughters Yididia Getachew, Dibora Getachew and my son Nahom Getachew.

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LIST OF ABBREVIATIONS

Bwta-	Body weight after infection
Bwtb-	Body weight before infection
Bwtd-	Body weight difference
CfL-	Control female Local
CfR -	Control female Rhode Island Red
CI-	Confidence Interval
CmL-	Control male Local
CmR-	Control male Rhode Island Red
CSA-	Central Statistics Authority
EARO-	Ethiopian Agricultural Research Organization
FAO-	Food and Agricultural Organization
LSC-	Local strain chicken
masl-	meters above sea level
OPG-	Oocyst per gram feces
PCVa-	Packed Cell Volume values after inoculation
PCVb-	Packed Cell Volume values before inoculation
PCVr-	Packed Cell Volume value reduction
PI –	Post infection
r –	Correlation
RIR-	Rhode Island Red
SE-	Standard error
TfL –	Test female Local
TfR-	Test female Rhode Island Red
TmL-	Test male Local
TmR-	Test male Rhode Island Red
TOP-	Total oocyst production

ABSTRACT

The objective of this study was first to investigate the prevalence of poultry coccidiosis and to identify the coccidial species occurring in the study area on local strain and Rhode Island Red breed chicken. The duration of the study was from September up to December, 2003 and the sample size for local strain and RIR breed was 160 and 31 respectively. The study involved questionnaire survey, fecal examination, necropsy examination and identification of coccidial species based on their morphology, predilection site in the intestine and sporulation time. Frequency detection of oocyst in the fecal samples from RIR breed and local strain chicken was 80.65% and 61.25% respectively. This finding indicated that coccidial infection in RIR breed was significantly higher than in local strain chicken ($p < 0.05$). The lesion score and mean oocyst output per gram feces was also considerably higher in RIR breed than in local strain chicken ($p < 0.05$, $P < 0.001$ respectively), which may be the difference due to management system and breed. Clinical coccidiosis occurrence in RIR breed and local strain chicken was 22.58% and 12.25% respectively. There was no statistically significant difference in clinical coccidiosis occurrence between the two genotype chickens and system. *Eimeria* species identified in descending order of their occurrence were *E. tenella, E. acervulina, E. necatrix, E. maxima* and *E. mitis*. Mixed infections were the predominant in both production systems. *E. mitis* was tentatively diagnosed for the first time in Ethiopia. The second study was experimental study was conducted to compare the natural resistance of local strain and RIR breed chicken to acute infection with *E. tenella* field isolate. The resistance was assessed based on measuring and analysis of the parameters; oocyst production, body weight gain/loss, lesion score, packed cell volume and morbidity rate. A total of 218 chicks were assigned for the experiment out of which 141 local strains and 77 RIR breed were randomly allocated into the experimental test and control groups. Sex difference within each genotype was also taken in to account. The test groups were inoculated with 150,000 sporulated oocysts of *E. tenella* per bird suspended in 1ml distilled water and given per *os* directly in to the crop. Body weight and packed cell volume measurements were taken for all birds twice, on day 0 and 8 post infection. The birds were necropsied 8 days post infection for lesion score. The morbidity rate in RIR breed was lower ($p < 0.001$) than the local strain chickens and no mortality during the patent period. Moreover, body weight depression in RIR breed was significantly lower ($P < 0.05$) than the local strain chicken. However, lesion score and oocyst production was significantly higher than the local strain chickens ($p < 0.001$, $p <$

0.001). This indicated RIR breed demonstrated better resistance to overcome the effects of the disease. Differences in natural resistance between males and females were not significant. However, under experimental infection there were some variations to the effects of the disease that females showed significantly higher body weight depression and larger oocyst production than males in both genotype chickens.

Key Words: Coccidia, *E. tenella*, natural resistance, prevalence, infection, Rhode Island Red breed, Local strain chicken and Tiyo Wereda.

1. INTRODUCTION

Poultry production in its general term includes the production of domesticated birds such as chicken, turkeys, ducks, geese and others, which are mainly kept for production of egg and meat. Among these chickens are the most important species, adapted globally to different ecological condition where human being lives and contribute a significant role in supplying animal origin protein to improve human nutrition (EARO, 2000). In developing countries poultry production offers an opportunity to feed the fast growing human population and to provide income for resource poor farmers. Moreover, poultry in many parts of the modern world is considered the chief source of not only cheaper protein of animal origin but also of high quality human food. The total poultry population in Ethiopia is estimated to be 56.5 million (CSA, 1998). The total national egg and poultry meat production is estimated to be 78000 and 72300 metric tones respectively, of which local birds, kept under the traditional systems of production, contribute 98.5% and 99.2% (EARO, 2000). It is estimated that the per capita egg and chicken meat consumption are about 57 eggs and 2kg per annum respectively. Poultry accounts for 15% of the total per capita meat consumption in Ethiopia (FAO, 1993). 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).

Coccidia infections in chicken cause greater financial losses than in other domesticated birds. It is caused by the genus *Eimeria* and nine species are known to occur in chicken, which are widely distributed throughout the world (Reid, 1978). *E. tenella* and *E. necatrix* are the most pathogenic species. *E. acervulina, E. maxima* and *E. mivati* are common and slightly to moderately pathogenic; *E. brunetti* is uncommon but pathogenic when it does occur. *E. mitis*, *E. praecox* and *E. hagani* are relatively non-pathogenic species (Soulsby, 1982; Lillehoj and Trout, 1993)).

Poultry coccidiosis is the term applied to the disease condition caused by infection with one or more species of coccidia. But coccidiasis indicates the condition of light infection of

pathogenic species of *Eimeria* or non-pathogenic species which reveal absence of clinical or sub clinical coccidiosis (Reid, 1978). The species of coccidia identified in Ethiopia are *E. tenella*, *E. necatrix*, *E. maxima* and *E. acervulina* (Methusela, 2001; Ashenafi, 2000). *E. mivati* was also reported (Guale, 1990).

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 a bird at one time, the pathogenicity of the *Eimeria* species, the age of the infected chicken and the management system (Reid, 1990). *E. 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.

In general, the losses caused by coccidiosis without including the sub clinical coccidiosis are estimated to be 2 billion USD throughout the world (O'Lorcain *et al.,* 1996). Losses due to sub clinical forms of the disease are heavy and can't be estimated (Gordon and Jordan, 1982). Quantitative losses due to coccidiosis in Ethiopia are not well documented, but (Methusela, 2001) 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.

The extensive use of anticoccidial drugs has led to the development of resistance strains, which ultimately resulted in reduced activity of the drug against the agent (Graat *et al.,* 1996;

Reid, 1990). The other approaches, alternative to drugs to minimize the losses due to coccidiosis are induction of protective immunity through vaccination and the use of naturally resistant birds (Pinard-van Der Laan *et al.,* 1998). Disease control based on host natural resistance becomes an attractive alternative approach (Zhu *et al.*, 2000). Several authors have reported the existence of genetic variation in resistance to coccidiosis among breeds and strains (Lillehoj, *et al.,* 1989; Lillehoj and Trout, 1993; Bumstead, *et al.,* 1991). Selection of poultry for natural resistance to coccidiosis is a promising method of control (Whiteman and Bickford, 1989). However, this method requires direct challenge and therefore cannot be a routine procedure for commercial lines, for economical reasons (Pinard- Van Del Laan *et al.,* 1998).

Natural resistance to coccidiosis can be considered as a quantitative trait which is controlled by multiple gene actions. Unlike other quantitative traits, such as growth and egg production disease resistance is usually difficult to measure directly. Instead, the most commonly measured parameters for the evaluation of natural resistance are body weight gain, lesion score, feed conversion and oosyst shedding after chickens are inoculated with an equal amount of oocysts to reflect the resistance or susceptibility status (Conway *et al.,* 1993; Pinard- Van Der Laan *et al.,* 1998; Zhu *et al*., 2000).

Preliminary studies on the prevalence of coccidiosis done in the past have shown that both clinical and sub clinical coccidiosis have been occurring with low prevalence rate in the local strain chicken kept under the backyard production system than in the commercially oriented production systems (Guale, 1990; Ashenafi, *et al.,* 2004). It is logical to speculate that management difference could alter the prevalence rate. However, the natural resistance of the local strain chicken to coccidiosis should be further studied to know whether the low prevalence was due to only management system or the potential natural resistance of these strains to coccidial infections. There was no study done in our country to identify the presence or absence of any potential natural resistance possessed in this local strain chicken against coccidiosis.

Thus, the objective of this study is to conduct prevalence study of poultry coccidiosis in Tiyo Wereda, Arsi Administrative Zone of the Oromia Regional State, which may represent the mid and highland agro-ecology of the zone. The study included the indigenous strain chicken under free- range management system and the Rhode Island Red breed kept under large-scale

deep litter management system to observe the importance of coccidiosis in these genotype chickens and management systems. The species of Eimeria occurring in the area were identified and ranked based on their frequency of occurrence. The other objective is to compare the natural resistance of local strain chicken and Rhode Island Red breeds to *E. tenella* acute experimental infection.

2. EPIDEMIOLOGY

2.1. Etiology

The majority of the coccidia of importance in domestic animals belong to the genus Eimeria. The genus Eimeria is one of the 25 genera recognized under the family Eimeriididae of the subphylum Apicomlexa. Coccidiosis could be regarded as ubiquitous in poultry management (Soulsby, 1982). Chickens are the natural host of nine Eimeria species among which six species namely *E. tenella, E. necatrix, E. brunetti, E. maxima E. acervulina* and *E. mivati* produce moderate to severe intestinal or cecal lesions. *E. praecox, E. hagani* and *E. mitis* do not cause significant lesions. (Reid, 1978; Lillehoj and Trout, 1993). Coccidia exhibit marked degree of host specificity (Becker, 1962).

The following characteristics are useful in species identification and they are illustrated in (Figure- 1) (Conway and McKenzie, 1991).

1. Zone of intestine parasitized
2. Nature of macroscopic lesions
3. Oocysts size, shape and color
4. Minimum sporulation time
5. Minimum prepatent period
6. Schizonts size and area in which the parasite develops
7. Location of the parasite within the epithelial cells and
8. Cross-immunization properties.

TABLE 1. Differential characteristics for 9 species of chicken coccidia (Reid 1968)

DIAGNOSTIC CHARACTERISTICS IN RED

CHARACTERISTICS	*E. acervulina*	*E. brunetti*	*E. hagani*	*E. maxima*	*E. mivati*	*E. mitis*	*E. necatrix*	*E. praecox*	*E. tenella*
Zone / Parasitized		moves down			moves down		Large schizonts no oocysts; unsporulated oocysts		
Macroscopic Lesions	light infection transverse, whitish bands of oocysts heavy infection plaques coalescing thickened wall	coagulation necrosis mucoid, bloody enteritis	pinhead hemorrhages petechiae	thickened walls, mucoid, blood-tinged exudate, petechiae	light infection rounded plaques of oocysts heavy infection thickened walls coalescing plaques	no lesions, 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
Microscopic Characteristics in µm / Oocysts Redrawn from Originals	20 µm		none available						
Length x Width / **Length = Width =**	AV = 18.3 x 14.6 (µm) 17.7-20.2 13.7-16.3	24.6 x 18.8 20.7-30.3 18.1-24.2	19.1 x 17.6 15.8-20.9 14.3-19.5	30.5 x 20.7 25.5-42.5 16.5-29.8	15.6 x 13.4 11.1-19.9 10.5-16.2	16.2 x 16.0 14.3-19.6 13.0-17.0	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	22.6 x 19.0 19.5 26.0 16.5 22.8
Oocyst Shape and Index—Length/Width	ovoid 1.25	ovoid 1.31	broadly ovoid 1.08	ovoid 1.47	ellipsoid to broadly ovoid 1.16	subspherical 1.01	oblong-ovoid 1.19	ovoid 1.24	ovoid 1.16
Sporulation Minimum Hr	17	18	18	30	12	18	18	12	18
Schizont, Max in µm	10.3	30.0		94	17.3	11.3	65.9	20	54.0
Parasite Location In Tissue Sections	epithelial	2nd generation schizonts subepithelial	epithelial	gamelocytes subepithelial	epithelial	epithelial	2nd generation schizonts subepithelial	epithelial	2nd generation schizonts subepithelial
Prepatent Period-Hr	97	120	99	123	93	138	99	84	138

Figure 1. Differential Characteristics for the 9 species of Chicken Eimeria.

Source: Reid, W. M. (1978)

Oocyst size and color are useful in identification of *E. maxima.* Overlapping sizes of other species require measurements. Oocysts shape differs from species to species. Edgar and Seibold, (1964) cited by (Reid, 1978) classified that *E. tenella*, *E. maxima, E .acervulina* and *E. brunetti* as being ovoid; *E. necatrix* as oblong ovoid; and *E. hagani* as broadly ovoid. However, since these descriptive terms are subjective, "Shape index" determined by dividing the mean length by the mean width may be more useful.

So far different investigators which have carried out studies on poultry coccidiosis in Ethiopia have found up to 5 (five) species. Guale, (1990) has identified three species namely *E. tenella*, *E. necatrix* and *E. acervulina*. However, Ashenafi, 2000 and Methusela, 2001 identified the above three species and additionally *E. maxima*.

2.2. Life Cycle of Coccidia

The coccidia exhibit a complex life cycle, comprised of both intracellular and extracellular stages. The intracellular reproductive process has asexual and sexual reproduction taking place inside the bird and the extra cellular stage is maturation of the oocysts outside the bird by sporulation (Reid, 1978).

The typical life cycle of coccidia consists of three phases sporogony, merogony (schizogony) and gametogony (Reid, 1978; Lillehoj and Trout, 1993). During sporogony, which occurs outside the host, four sporocysts each containing two sporozoites are formed (Figure 2-t and u) (Gordon and Jordan, 1982). Sporulated oocysts, when ingested by susceptible hosts, initiate the infective cycle. After ingestion sporozoites invade the intestinal epithelium and round up to form a trophozoite followed by nuclear division to form an immature meront (schizont) by which the merogony stage commences. A varying numbers of merozoites are being produced asexually by multiple fission from each meront. *E. tenella* has 2-3 generations of merogony. The first generation schizont measures up to 54µm in diameter and may contain up to 900 first generation meroziotes. This mature schizont ruptures into the lumen of the crypts of the cecal glands 3 days post infection (P.I) and the merozoites penetrate other epithelial cells to form young second generation schizonts. Colonies of the second generation schizonts mature by day 4 P.I and release about 300 second generation merozoites into the lumen of the cecum. When large numbers of second generation schizonts are involved, a massive hemorrhage into

the cecal lumen may be evident at about day 4 of P.I (Soulsby, 1982). Second generation merozoites penetrate new epithelial cells and initiate either third generation of schizonts or the gametogonous cycle; the majority undertaking gametogony cycle (Figure 2- a up to h).

Gametogony starts when merozoites invade cells and develop into either macrogamonts or microgamonts. The former gives rise to a single macrogamete where as the latter undergoes multiple divisions resulting in the formation of numerous flagellated microgametes. Fertilization occurs when the microgamete invades cells containing macrogamete; a wall forms as the oocysts mature (Figure 2- i up to s) (Conway and McKenzie, 1991; Lillehoj and Trout, 1993). Although the general live cycle is the same for all Eimeria, host specificity, site of development, patent and prepatent periods and pathogenicity vary between species (Becker, 1962). Only a small proportion of the millions of oocysts produced by a bird survives and become infective. Essential conditions for the survival are sufficient moisture, oxygen and suitable temperatures.

Sporulation of *E. tenella* is most rapid at 28^0C slow down at 20 or 32^0C and ceases at 8 or 37^0C. Since oocysts are killed within 10 minutes of exposure at 55^0C, heat furnishes a convenient laboratory method of sterilization. Similarly, oocysts are killed by freezing or even if a few ice crystals developed in suspension fluid (Reid, 1978). Oocyst survival time is greatly extended in the presence of high humidity. The optimum level of temperature and relative humidity required for sporulation of oocysts are 29^0C and 50-75% respectively (Urquhart *et al*., 1987). In vitro cultivation of *E. tenella* has been done in a variety of cell types to various stages of development from the sporozoite stage (Soulsby, 1982).

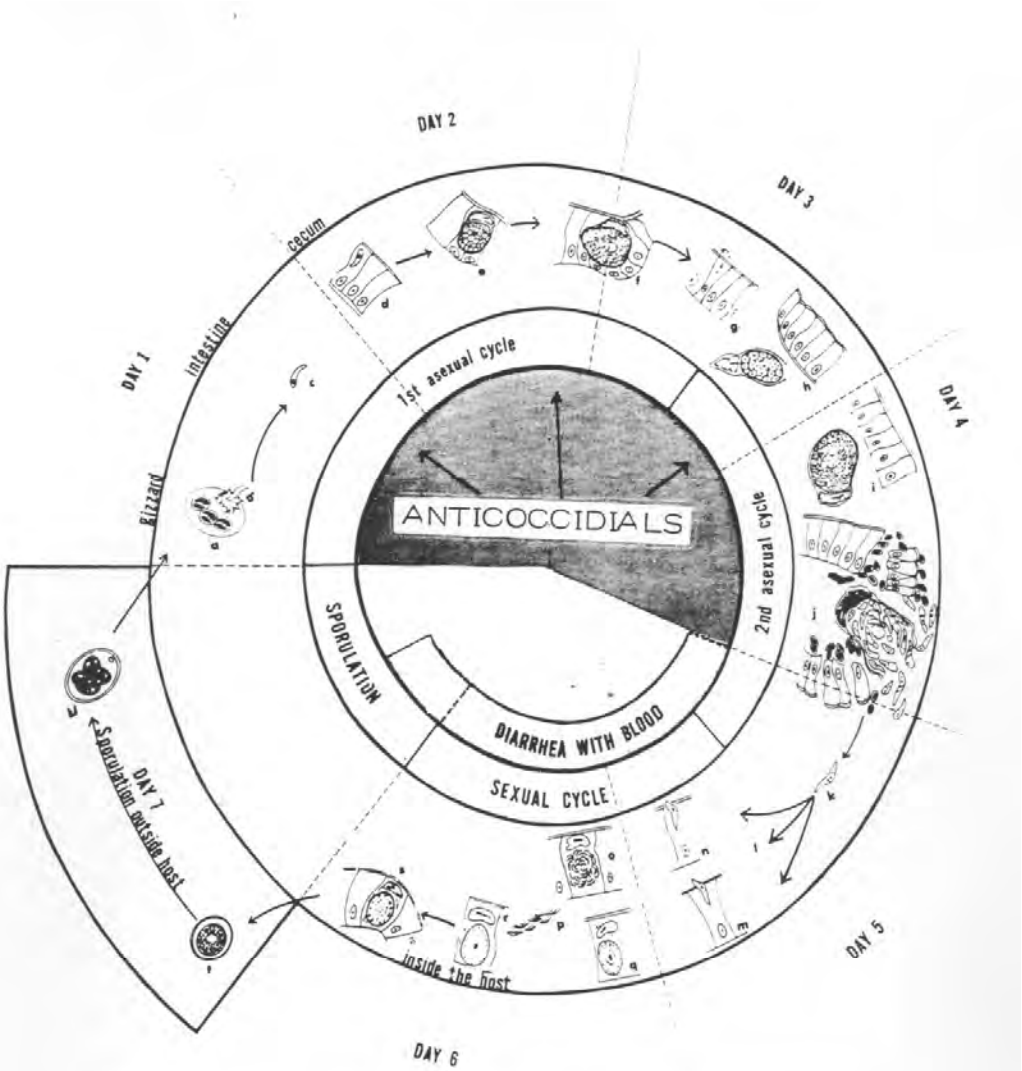


FIG. Life cycle of *E. tenella*: *(a)* sporulated oocyst; *(b)* sporozoite being liberated from oocyst and sporozoite; *(c)* sporozoite; *(d)* trophozoite parasitizing an epithelial cell; *(e)* early schizont; *(f)* mature first-generation schizont; *(g)* first-generation merozoite parasitizing another epithelial cell; *(h, i)* second-generation schizonts; *(j)* rupture of second-generation schizont; *(k)* second-generation merozoite may again parasitize other epithelial cells *(l)* for a third asexual cycle, or may parasitize an epithelial cell *(m)* to become a female gametocyte *(q)*; merozoite parasitizing an epithelial cell *(n)* and becoming a male gametocyte *(o)*; *(p)* liberated microgametes unite with macrogamete *(r)*, which develops into oocyst *(s)* and is liberated in the feces by host *(t)*; sporulation *(u)* of oocyst occurs in outside environment.

Figure 2: Life cycle of *E. tenella* and the Effective Anti-Coccidial Action Period. Source: Reid, W. M. (1978)

2.3. Transmission and Occurrence of Infection

Ingestion of viable sporulated oocyst is the only natural method of transmission. Both diseased and recovered chickens may continue to shed oocysts. There was a steady escape of oocysts demonstrated by the occasional finding of oocysts in the cecal contents up to 7.5 months (Becker, 1962). But birds stricken with severe coccidiosis may die before oocysts have completed their development (Reid, 1978; Conway *et al.*, 1991).

Human beings are the main mechanical transmitters in disseminating oocysts, which could be carried over by manure clinging to shoes or by utensils carried about from one pen to another. Flies, beetles, cockroaches, rodents, pets and wild birds have also been incriminated as mechanical vectors (Reid, 1978). Oocysts may survive as long as 86 weeks in shaded soil. But sunlight assists in destruction of oocysts. Incubator temperature held for several days will kill oocysts, so there is no danger of hatchery transmission to the baby chicks. Oocysts are so resistant to disinfectants that they survive stringent attempts to kill them (Becker, 1962).

In chicken reared outdoors under range conditions, coccidiosis outbreaks are common in the spring and summer than cooler fall and winter conditions. If confinement rearing is practiced outbreaks may occur at any season of the year. However, there is some evidence of more rapid cycling in spring and summer than in fall and winter (Reid, 1978; Soulsby, 1982). Under traditional backyard production the prevalence rate of 11% and 25.8% sub clinical coccidiosis have been reported in some parts of Ethiopia (Guale, 1990 and Ashenafi, *et al.*, 2004). Moreover, the latter investigator also recorded 15.8% clinical coccidiosis.

The increased use of cages for laying hens has greatly reduced the number of outbreaks that occur in these valuable birds (Reid, 1990). In cage system there is no contact between the birds and their feces, thus transmission would be minimal. Chickens, which were kept under cage system, had only 1% infection (Guale, 1990). It was presumed that the possible causes of infection in cage birds are human beings and may be from feed processing manufacturers too. However, in deep litter poultry houses, which offer optimal condition of temperature and humidity for oocyst sporulation, the risk of infection is further increased (Urquhart *et al.*, 1987; Becker, 1962). Depending on the degree of vigilant control of the disease and the age of the chicken exposed to the risk, a prevalence rate as low as 6.8% and as high as 80.3% has been observed in different urban and peri-urban poultry farms in Ethiopia (Methusela, 2001).

2.4. Pathogenesis and Clinical Signs

Infection with single species of coccidium is rare in natural conditions, and mixed infections are common. Nevertheless, in many outbreaks the clinical entity can be ascribed principally to one species or occasionally a combination of two or three (Soulsby, 1982). The clinical disease is dependent on the number of oocysts ingested by individual birds. If the environment hygiene is poor, this number may be very large which is particularly true for *E. tenella* that have high biotic potential. But in very light doses no clinically recognizable symptoms may occur and thus, the morbidity and mortality increase in proportion to the size of the dose ingested (Becker, 1962; Soulsby, 1982).

Many of the worst coccidial outbreaks occur at the age of 4-8 weeks. Herrick ott and Holmes, 1936 cited by Becker, (1962) made a study of experimental infections in which they demonstrated the heaviest mortality of 72% occurring in chicks 1 month old as well as the greatest decrease in RBC 60%. Mortality and RBC decrease were also heavy at the age of 1.5 to 2 months, while older birds (3,4,7,10 and 15 months) mortality was low or lacking, though the drop in RBC counts ranged from 29%-46.8%.

2.4.1. *E. tenella*

E. tenella is one of the most common and pathogenic coccidia of domestic poultry, which is distributed worldwide. It develops in the cecum and adjacent areas of the digestive tracts and causes "bloody" or cecal coccidiosis. Because of the sudden onset of mortality in severe infections, this species has been the most feared by poultry producers (Reid, 1978; Soulsby, 1982).

On a flock basis the first sign of coccidiosis becomes noticeable at about 3 days after infection. The first sign of morbidity is a refusal to feed and drink. Chicken shows droop; huddle to keep warm and diarrhea. Blood loss from cecal lesions and characteristic odor may be noted shortly before mortality begins. The greatest hemorrhage occurs on the day 5 and 6 of infection, and by the day 8 or 9 the bird is either dead or on the way to recovery. Mortality is highest between the fourth and sixth day, death sometimes occurring unexpectedly due to excessive loss of blood (Soulsby, 1982). The recorded durations for the occurrence of the above events could vary in 24 hours up and down (Williams, 2001). Coccidiosis is a self-

limiting disease. Regeneration of the epithelium and glands is completed by the 10^{th} day in light infections; healing in severe infections may take 3 weeks (Witlock *et al.*, 1975 cited by Reid, 1978). In recovered birds a chronic illness may develop as a result of a persistence cecal core. RBC count and haematocrite value may decrease as much as 50% on the 5^{th} and 6^{th} day of infection. The normal weight are never fully regained during the growing period (Conway and McKenzie, 1991)

Cecal coccidiosis due to *E. tenella* most frequently occurs in young birds especially those at 4 weeks of age. Older birds are generally immune as a result of previous infection (Soulsby, 1982). In general, clinical cecal coccidiosis is produced only when heavy infections are acquired within not exceeding 72 hours period of time (Soulsby, 1982). Conway *et al*., (1993) in 9 day-old male broiler chickens have investigated the number of oocysts required to produce clinical disease. It was found that plasma carotinoids, PCV and lipids were significantly depressed at the oocyst dose of 10^3 and higher per birds. Plasma protein was significantly depressed at 10^2 and higher doses. Mortality due to cecal coccidiosis was 5% and 25% at 10^4 and 10^5 oocysts per bird respectively. The weight and feed gain are also significantly depressed at these doses. Lesion scores increased significantly with increasing oocyst doses but the distribution at the two highest doses (10^4 and 10^5) were not significantly different from each other.

2.4.2. *E. necatrix*

It is one of the most important pathogens of the small intestine of poultry, which is also distributed worldwide. The sexual development occurs in the small intestine but the gametogony cycle in the cecum (Soulsby, 1982). Massive dilation or "ballooning" of the middle intestine suggests the presence of *E. necatrix* coccidiosis although *E. maxima* may, also produce the same effect. Presence of Schizonts that may be as large as 66 μm in diameter is diagnostic for *E. necatrix* (Conway and McKenzie, 1991). Infected birds show listlessness, drooping wings, humped back, and dehydration. Mortality begins on the 5^{th} day, shows greatest severity on the 7^{th} and extends to the 12^{th} day. The principal lesions are found in the middle third of the small intestine and in severe cases marked congestion, bleeding and necrosis occur on the mucosal surface of the intestine. The appearance of the whitish yellow

plaques containing schizonts is the pathogenomonic lesion. A focal type parasitic granuloma reaction is induced by only this species of coccidia (Reid, 1978). *E. necatrix* is poor in biotic potential.

2.4.3. *E. acervulina*

E. acervulina is very commonly seen worldwide in distribution and less pathogenic than the two previous species. It is usually responsible for subacute or chronic intestinal coccidiosis in older birds and chicken at the point of lay. It mostly localizes and develops in the epithelia cells of the duodenum and jejunum (Soulsby, 1982).

Within recent years especially in large poultry establishments, the significance of this species as a pathogen has increased steadily. The biotic potential is great, the prepatent and sporulation periods are short, and very large numbers of oocyst may accumulate in the environment. The severity of the disease depends directly on the numbers of oocysts to which the bird is exposed (Reid, 1978). The clinical signs consist of weight loss and watery whitish diarrhea. At postmortem grayish white, pinpoint foci or transversely elongated "ladder like' areas are visible from the serosa surface of the duodenum (Conway and McKenzie, 1991).

2.4.4. *E. maxima*

It is common and has a worldwide distribution. Developmental stages occur in the middle small intestine, which shows hemorrhagic enteritis associated with thickening of the intestinal wall and some ballooning. Oocysts are large ovoid with distinct golden color that is discernible by comparing oocysts of other species (Conway and McKenzie, 1991).

This species should be classed as moderate to severely pathogenic. In severe cases, there is extreme emaciation, pallor, depigmentation of feathers and anorexia. Morbidity readily measured by weight loss in artificial infections. Light infections may induce depigmentation and poor carotinoid absorption with economic consequences where local preference brings premium prices for deep pigmentation (Ruff *et al.*, 1976) cited by Reid, (1978). Rapid development of immunity frequently aids in control of *E. maxima* coccidiosis.

2.4.5. *E. brunetti*

It is uncommon, although markedly pathogenic when it does occur. Developmental stages are in the lower small intestine cecum and cloaca (Soulsby, 1982). In severe cases the lesions are characterized by coagulation necrosis appears on days 4-7 producing a caseous eroded surface over the entire mucosa and the condition is typically a rectal coccidiosis. Hemorrhagic catarrhal exudates appear 4-5 days after experimental infection (Reid, 1978). The dropping is fluid, may be blood stained and a mortality rate of 10% has sometimes been reported from this species alone. Sub clinical infections are often over looked. But in cases where lesions are almost invisible, it can induce weight losses of economic importance (Reid and Johnson, 1974) cited by Reid, (1978).

2.4.6. Other Species

E. mivati occurs in the anterior half of the small intestine and recently described as one of the chicken coccidia. Regarding its pathogenicity it seldom produces heavy mortality. But experimental infection with 5-10 million oocysts can produce mortality. Birds show listlessness, anorexia, ruffled feathers and watery diarrhea 4 days after infection. Reduction in egg production has been reported with poor response to anti-coccidial treatment (Soulsby, 1982).

E. mitis, E. praecox and *E. hagani* are generally considered as less or mildly pathogenic with apparently no significant lesions (Conway and McKenzie, 1991). The developmental stages of all are from the anterior portion up to the middle small intestine. Infection usually occurs in the flock as the sub clinical form, which has little or no economic importance and chicken may develop sufficient immunity after exposure to the agents. However, lack of immunity and repeated infections have been reported with *E. mitis* causing loss in egg production and body weight gain (Fitz-con and Edgar, 1992).

2.5. Histopathological Changes of *E. tenella*

The general effects include changes in the cellular kinetics and morphology of the villi. The pathological changes are mainly due to the second generation schizonts (Soulsby, 1982). Petechial hemorrhages occur during the first three days and noticeable lesions consisting of marked hemorrhagic spots appear on the fourth day. By the fifth or sixth day the caeca are dilated, the contents containing unclotted and partly clotted blood, schizonts and merozoites. By the seventh day the cecal contents have become more consolidated and caseous, adherent to the mucous membrane. By the eighth day the consolidated caseous plug completely fills the lumen of the cecum. The cecal cores detach from the mucous membrane by 8-10 days and may be shed in the feces. At this time the cecal wall is still thickened but it has lost its intense hemorrhagic appearance and regeneration of the mucosa occurs and the walls contracts (Soulsby, 1982).

The cecal lesion consists of initially both cryptal and absorptive epithelial cells destruction and submucosa edema (Reid, 1978). In primary infection, numbers of heterophils and mast cells were increased during the acute inflammation process which indicates mast cells play a role as primary inflammatory cells (Petrone *et al*., 2002). Heterophils predominated when necrosis was extensive; otherwise, mononuclear cells were the main inflammatory cells (Mesfin *et al*., 1978; Reid, 1978).

The sites of the development stages of schizonts and gametocytes in the epithelial cells of the intestine or cecum with reference to the nuclei of the epithelial cells is directly associated with the severity of lesions produced in the intestine (Table-1) (Reid, 1978; Soulsby, 1982). More pathogenic species develop deep in the epithelial cells of the intestine beneath the nucleus in their developmental stages.

Table 1. The location of different developmental stages in of Eimeria the intestinal epithelium

Eimeria species	**Schizonts**	**Gametocytes**
E. tenella	B.N	**B.N**
E. necatrix	S.N	**B.N**
E. acervulina	S.N	**S.N**
E. maxima	S.N	**B.N**
E. brunetti	S.N	**S.N/ B.N**
All other species	**S.N**	**S.N**

S.N Superficial to the epithelial cell nucleus

B.N. Beneath to the epithelial cell nucleus

Source: Reid, 1978 with some modification.

3. DIAGNOSIS

A presumptive diagnosis is usually possible after a necropsy examination of recently killed birds and microscopic examination of smear preparations. Confirmation may require cross-immunization experiments (Conway and McKenzie, 1991). 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 number 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).

3.1. Gross Lesions

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 (Johnson and Reid, 1970) cited by Conway and McKenzie, (1991). 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).

3.1.1. *E. tenella* +1: A few scattered petechiae which are reddish or purple in color are seen on the unopened cecum. Less apparent petechiae are observed on the opened cecum. There is no thickening of the cecal wall and not extend to the adjacent intestinal portion than caeca. Mild clinical signs may show in infected chickens.

3.1.2. *E. tenella* +2: Petechiae on the serosal surface are somewhat more numerous. Bleeding which appears on the day fifth and seventh of infection is more marked on the mucosal surface than in 1+ score. Except for the presence of some blood, the cecal contents are normal. Another more reliable characteristic in judging severity is the amount of thickening of intestinal wall, which is slight in this case. Clinical signs are apparent in infected chickens with this degree of infection.

3.1.3. *E. tenella* + 3: Bleeding is more severe with clotting appearing in the distal end of the pouch. The clot becomes hardened as the sloughed mucosal surface joins the bloody material to form a core. The absence of normal cecal contents since the caeca has become practically unfunctional. Marked thickening of the cecal wall occurs. The unopened cecum serosa shows the petechiae as coalesced and eroding the entire surface. Huddling, chilling and bloody droppings constitute clinical signs.

3.1.4. *E. tenella* + 4: Severe bleeding, a much-thickened cecal wall and eroding of the mucosal surface show up on the 5^{th} day of infection. The unopened cecum is distended with blood at the distal end but contracted and shorted. Chickens huddle and sometimes let out a high-pitched call and cease feeding and drinking. Death may come suddenly beginning on the 5^{th} day, reaching the greatest number on the 6^{th} and extending through the 7^{th} to the 10^{th} day of infection.

By the 6th and 8th day the cecal core is hardened and may persist for another week or more. The core may take on a more whitish cast with a huge accumulation of sloughed mucosal surface. Purple areas denoting the presences of gangrene and rupture of the cecal wall may occasionally occur at this stage. Dead birds are scored +4 (Conway and McKenzie, 1991).

3.2. Microscopic Examinations

Parasitic stages demonstrated in fresh smear preparations are usually adequate in confirming a diagnosis. A standard parasitological technique for demonstration of the parasite from intestinal mucosa scraping can be used efficiently (Reid, 1978; Conway and McKenzie, 1991). However if no suspicious lesions have been noted on the intestine, select and take scrapings from the following parts of the intestine.

1. The duodenal loop below the entrance of the bile duct
2. The mid gut region near the yolk sac diverticulum
3. The lower intestinal area just above the union of cecal pouches and
4. An area from the middle of the cecum
5. The rectal area (Reid, 1978; Conway and McKenzie, 1991).

Oocysts are the most commonly recognized stages of the lifecycle and may be present in large numbers. However, because they develop late in the cycle they may be entirely missing in the presence of severe coccidiosis. Presence of distinctive schizonts of *E. necatrix* from the mid gut area is pathognomonic for this species (Conway and McKenzie, 1991).

Oocyst size and shape are less useful as diagnostic characteristics in chickens than in many other animals. Because they are similar except with *E. maxima*. Measurement of a number of oocysts (10 suggested) using an ocular micrometer is required. Length and width must be determined by selecting a side and not an end view of the oocyst. Thick fecal smears frequently prevent securing the necessary side view of the oocyst. Average length, average width, range in length, and range in width should be determined (Reid, 1978). In critical studies in species identification additional techniques other than microscope examinations are needed:

- Time required for sporulation and prepatent period

- Cross-immunity tests
- Location of the parasite in relation to the host epithelial cells nuclei and
- Tissue culture or egg embryo developmental differences may prove useful in identification of some species (Reid, 1978).

4. IMMUNITY

The nature of the immune mechanism that protects against coccidiosis appears to differ from the type demonstrated for viral and bacterial organisms. With coccidian localized tissue immunity persists in the intestine after all evidence of circulating antibodies has disappeared. Moreover, the serum antibodies do not appear to be the primary mechanism furnishing protection. Because the host may show strong resistance to challenge inoculation after these antibodies have disappeared (Davis*, et al.*, 1978). Thus, Davis *et al.,* (1978) suggested that the intestinal secretory IgA systems play an essential role in the protective immune response to *E. tenella*. Furthermore, the invasive extra cellular stages should be particularly vulnerable to the effect of secretory IgA (Davis*, et al.*, 1978). Recent studies suggest that resistance to coccidiosis in subsequent infections is a function of cell-mediated immunity although the role of humoral factors has not been excluded (Bumstead *et al.*, 1989).

The second-generation schizonts are the principal stages responsible for the induction of the immunity (Soulsby, 1982). After an outbreak of coccidiosis most birds rapidly develop self-limiting immunity, which induces species-specific T cell- mediated immunity (Byrnes *et al*., 1993). After experimental *E. tenella* infection, Interluekin-2 production increased significantly at 7 days post- primary infection and 2 days post- secondary infection. This result confirmed that cellular immunity elicited during an anamnestic response to coccidial re-infection is mediated at least in part, by IL-2 (Miyamoto *et al.,* 2002). Resistance may be partial or complete depending on the species of coccidia and number of subsequent re-exposure (Stiff and Bafundo, 1993). Repeated daily dosage of single or 5 oocysts over a period of 20 days produced strong immunity to E. *maxima*, E. *acervulina* and E. *tenella* than a single inoculation of the same or a larger dose. Single severe infections do not confer 'solid' immunity but often prevent mortality (Nakai *et al.,* 1992; Graat *et al.*, 1996).

4.1. Duration of Immunity

The resistance, which is developed from a previous infection, will protect birds from subsequent exposure to that species, but it does not induce immunity to another species (Soulsby, 1982). The duration of immunity developed under field flock management is usually of long period. Sufficient sporulated oocysts are usually present permit recycling and reinforcement if immunity levels begin to fall. This natural reinforcement of immunity may fail to occur if a flock is moved to new litter and a new location (Reid, 1978). However, investigators have disagreed on the duration of immunity if re-infection is completely prevented. Decrease in immunity levels as early as 42 days after initial immunization have been reported. But Edgre, (1968) cited by (Reid, 1978) indicated that using early exposure in floor-pen, found sufficient immunity to protect against economic losses for 6, 12, or 18 months.

Day-old-chicks are susceptible to coccidiosis but may be partially protected by parental immunity. Outbreaks that are common at 4-6 weeks of age seldom occur at less than 11 days of age. The popular view that older birds may develop strong resistance with age may be due to undetected immunity induced by accidental exposure to small number of oocysts, which is called naturally acquired immunity. Otherwise, unexposed adult birds remain highly susceptible to all species (Reid, 1978). Moreover, day-old-chicks exposed to coccidia (*E. tenella*) developed immunity in the same way as young birds (Stiff and Bafundo, 1993).

4.3. Natural Resistance to Coccidiosis

Several authors have reported the existence of genetic variation in resistance to coccidiosis among breeds and strains (Lillehoj and Trout, 1993). Resistance to coccidiosis in eight widely available inbred lines of chicken showed that there were considerable differences in susceptibility between the inbred lines (Bumsead *et al.,* 1991). Resistance to *Eimeria* infection seems to be due to both MHC and non- MHC associated genes. However, the relative magnitude in the contributions of these genes differs for the different coccidial species (Bumstead *et al.,* 1991). The resistance to *E. tenella* is also due to both MHC-associated and non- MHC- associated genes (Lillehoj *et al*., 1989).

Selection of poultry for natural resistance to coccidiosis is a promising method of control (Whiteman and Bickford, 1989). Chicken displayed a natural resistance and certain chicks rose from parents that were particularly resistant to *E. tenella* were approximately 100% more resistant than unselected chicks (Becker, 1962). Experimental mating involving resistance and susceptible F_1 and F_2 individuals showed that selective breeding was effective in establishing lines of chicken resistance or susceptible to cecal coccidiosis. It was concluded that resistance or susceptibility to cecal coccidiosis is controlled in a large part by multiple genetic factors that do not exhibit dominance and presumably act in an additive manner (Soulsby, 1982). However, so far this method of control has not been exploited

4.2. Concurrent Diseases

Experimental evidence shows that Marek's Disease may reduce resistance to coccidiosis by interference with coccidial immunity and the prognosis for flocks with combination of both coccidiosis and Marek's Disease is poor. The coccidiosis may not respond to either preventive or curative chemotherapy in normal way because the normal self-limiting immunity to coccidiosis fails to become established. Lack of vitamins A and K will also cause reduction in the general resistance of the bird, which causes the outbreak to be more severe (Reid, 1978).

5. FINANCIAL IMPACT OF COCCIDIOSIS

In all parts of the world where confinement rearing is practical coccidiosis represents a major disease problem (Reid, 1978). The increased crowding of chickens under mass production methods creates a favorable condition for the occurrence of coccidiosis. It is one of the top five chicken disease most frequently diagnosed in the field and laboratory involving 5-15% of all mortalities Sub clinical coccidiosis is more common than clinical coccidiosis. Losses due to this form of the disease are heavy and cannot be estimated (Gordon and Jordan, 1982). (Mayhew, 1932, 1934) cited by Becker, (1962) has found that birds inoculated during the 7^{th}, 13^{th} and 14^{th} weeks are definitely handicapped in that they do not regain the weight loss during an attack in the following three months as compared with the uninfected controls. In a later study he showed that hens developed from chicks inoculated at the age of 6-8 weeks laid 19.25% fewer eggs than the controls, and did not attain normal weight until 5 or 6 months after the attack. Furthermore, severely affected birds begin to lay eggs from 6-7 weeks later than the controls. `

A mild coccidiosis infection kept under control is not very harmful, and is actually necessary for creating immunity in replacement flocks and free ranging birds. However, a severe attack of the disease can cause weight loss, reduced egg production; morbidity and mortality (Reid, 1978; Lillehoj and Trout, 1993). An estimate based on guesswork has shown that $35-200 million dollar losses occur per year in USA due to coccidiosis. More than $45 million are being spent on prevention medication (Reid, 1978).

The estimation of financial losses due to coccidiosis in the industrial poultry farms and also the backyard poultry production in a comprehensive way has not been done in Ethiopia. Unlike the confinement rearing farms, free ranging backyard flocks are more often subjected to sub clinical coccidiosis. But sufficient information regarding the degree of this coccidiosis problem in this traditional management is not also available in the country.

6. TREATMENT, PREVENTION AND CONTROL

6.1. Treatment

In general, there are about 25 or more approved anticoccidial drugs (soulsby, 1982). Selection of the best is based on the ability of the drug to improve weight and feed conversion and to suppress the development of lesions (Reid, 1978). Anticoccidial agents (coccidiostats) show activity so early in the life cycle of the organism that few benefits are derived from treatment in later stages after symptoms appear (Figure-2). Early recognition of the disease may permit initiation of treatment before all birds have peaked up massive doses of oocysts. Delay of a few hours may be costly and delay of a full day may render treatment useless. For this reason, the preventive approaches have largely superseded treatment in the use of the drugs (Reid, 1978).

The emergence of drug resistance strains of coccidia presents a major problem. Continuous use of anti coccidial drugs leads to increased incidence of drug resistant strain development which, result in reduced activity of the drug against the agent (Graat *et al.*, 1996; Fitz-coy, 1992). Methods used to avoid the development of drug resistance include switching around the different classes of drugs: That is drug rotation and 'shuttle program', which is a planned switch of drugs in the middle of the growing period of birds (Soulsby, 1982).

The speed of resistant strain coccidia emergence in the field was put in order by (Reid, 1975) cited by Soulsby, (1982) as follows:

- Glycomide and Quinolines classes show rapid development of resistance.
- Nicarbazin and Monensin classes show very slow development of resistance.
- Amprolium, Zoalene, and Nitromide are seen to develop resistance slowly.
- The remaining classes of drugs are moderate in developing resistance. However, resistance strains have shown little cross-resistance between classes of anti coccidial drugs.

Precautions and restrictions on directions issued by the manufacturers need to be carefully followed. Generally, water is preferred medium for administering anti coccidial treatments, since medication is easily dispensed in water barrels than in feed (Reid, 1978).

6.2. Prevention and Control

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). However, the control and prevention option that one can follow depends on the management practice and the objective of the farm. For example, protection is more important with fast growing meat-type birds raised under floor-pen management than with egg producing types where immunity and caging alter the demand of anti coccidial drugs (Soulsby, 1982). On the contrary, if chickens are exposed to modest numbers of oocysts in their environment, they develop immunity to the species represented. Otherwise birds reared in complete isolation remains susceptible and may be subjected to severe outbreaks unless permitted to develop immunity by low level infection (Reid, 1978). Males *et al.*, (1992) revealed the effect of Diclazuril fed to broiler chicken at 1mg /kg of feed on the development of protective immunity against *E. tenella* experimentally that the drug did not significantly interfere with the formation of protective immunity.

6.3. Vaccination

Although several pharmaceutical companies and government agencies have research goals of producing a useful vaccination program, practical application appears to be some years away (Reid, 1990). Numerous vaccination strategies have been attempted to control avian coccidiosis. Mixtures of different live coccidial strains have been successfully used under continuous exposure to oocysts administration for 28 days in broilers (Stiff and Bafundo, 1993). The study demonstrated that regardless of the age of the broilers, all were capable of establishing complete protective immunity to *E. tenella, E. maxima* and *E. acervulina* within 25, 24, and 16 days respectively. However, the problem comes in controlling dosage with many species involved under field conditions presents a more difficult management problem.

Various attempts have been made to find attenuated strains of coccidia, which could be convenient for vaccination procedures. These have included embryo adaptation, development of precocious strains, X- irradiation and chemical treatment (Lillehoj and Trout, 1993). Practical application of recombinant vaccine to poultry industry is still remote (Lillehoj and Trout, 1993). However, Pogonka, *et al.,* (2003) have stated that single oral inoculation of recombinant *Salmonella Typhimurium* revealed it can induce specific antibody responses to heterologous *Eimeria* antigens in chickens, suggesting that recombinant *Salmonella* are a suitable delivery system for vaccines against Eimeria infections. The method used was *Salmonella Typhimurium* vaccine strains were used as antigen delivery system for oral immunization of chickens against *Eimeria* infections. The known *E. tenella* proteins SO7 and TA4 were sub cloned into plasmids pTECH2 vector system and allowed them to be expressed with the highly immunogenic fragment C of the tetanus toxin under control of the anaerobically inducible nirB promoter. Then stable recombinant bacteria were recovered containing the respective plasmids. After oral immunization the specific serum IgG antibodies against the SO7 and TA4 antigens were detectable by ELISA-2 two weeks after immunization and remained for at least 6 weeks.

Serial passage of strains in parasitized chicken embryos and selecting for precocious strains as originally described by (Jeffers, 1975) cited by Reid, (1990) have an advantage to produce genetically reproducible stable strains of attenuated coccidia. This method shows greatest promise for developing a true vaccine strain (Lillehoj *et al.*, 1993; Reid, 1990). Paracox vaccine has now been developed as a blend of oocysts of precocious lines of seven main pathogenic species of Eimeria from the chicken. The vaccine is now used in chickens kept for breeding purpose and egg- lying of non- caged system in Europe and many other countries (Shirley, 2001). The recently produced Paracox- 5 vaccine has extended forward the use of this vaccine for the production of broiler chickens. It is anticipated to eliminate the problems associated with coccidiostats resistant strains, and the need for drug withdrawal periods before the poultry can be sold for human consumption (Shirley, 2001).

The general method utilizes repeated passage of virulent coccidia, each time selecting for early produced oocysts. The change in normal life cycle is accompanied by marked reduction in prepatent time (at least one asexual stage is missing). This loss of replicating parasite leads to both the significant reduction in life cycle time and the dramatic phenotype of attenuation. However, each precocious line remains immunogenic since the first asexual stage is

apparently a key to the induction of a protective immune response have been retained (Lillehoj and Trout, 1993).

The sporozoites exposed to 12Rad. radiation are capable of inducing immunity against coccidiosis, as are non-irradiated oocysts (Jenkins *et al.*, 1993). Radiation attenuated *E. maxima* was effective in preventing weight depression of experimental challenge. However, the interesting finding was the immunization had less effect on the appearance of intestinal lesions.

6.4. Management Practice and Biosecurity

In most tropical countries like Ethiopia naturally ventilated poultry houses with controllable windows at the sides of the house is used. Proper ventilation brings in fresh air and takes out stale air. If the ventilation system is not effective, control of the poultry house with respect to temperature and relative humidity fails which will give an ideal condition for sporulation of the oocyst and this may lead to disease out break (Methusela, 2001).

Although it is difficult to control coccidiosis by biosecurity measures alone, it is still an important component of good management practice which will control coccidiosis and other diseases with their associated economic losses (Methusela, 2001). Biosecurity practice includes hygiene, disinfection and litter management. The feeding and watering system should be placed in a way that chicken can easily reach and adequate spaces are available for feeding and watering. Otherwise, contamination of feed and water with coccidia oocysts from chicken dropping is inevitable and hence increases the risk of infection and disease (Whiteman and Bickford, 1989). The birds should not be overcrowded. Prevention of damp areas in the house should be the practical emphasis of poultry producers. Leaking roofs or watering troughs should be adjusted; redistribute the litters frequently to avoid concentration of the oocysts at places such as feeding and watering troughs. Litter should be kept dry to 25-30% moisture (Soulsby, 1982). The house should be cleaned thoroughly before each new flock comes in. The walls and dirty floors should be washed out and disinfected (Whiteman and Bickford, 1989).

- The coccidial infection should be kept on the outside. Disinfect shoes before entering the chicken house. Keep wild birds and other animals out, separate older birds from younger birds (Zander, 1978).
- The poultry man should vigilantly watch for the decrease in feed consumption. A reduction in feed intake is a ‘sure sign’ of some thing is wrong (Anonymous, 1964).

7. MATERIALS AND METHODS

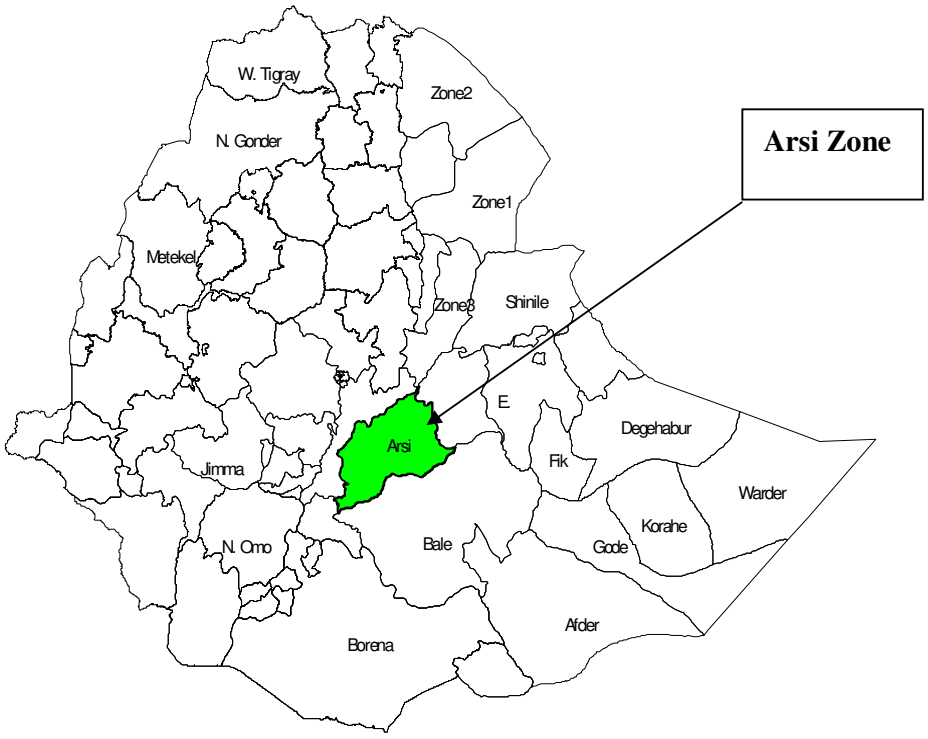
7.1. Study Area

Tiyo Wereda is located in Arsi Administrative Zone of Oromia Regional State (Figure 3 and 4). Asella is the main town for Arsi Zone and Tiyo Wereda too, which is located 175 km South-East to Addis Ababa at 07^0-56'-856N and 39^0-08'-260E, 2436 masl. Tiyo Wereda is one of the Twenty Weredas found in the Arsi Zone situated in the North Western part of the Zone (Figure-4). The Wereda has 65000 hectares land area with 18 administrative Kebeles. According to the agro-ecological condition, the Wereda has highland climate, which is 37% of the land area, midland, 52% and lowland 11%. Their respective land area is about 24,050 hectares, 33,800 hectares and 7,550 hectares respectively. Among the 18 Administrative Kebels, 7 Kebeles are located in highland, 9 in midland and 2 in lowland agro- ecological zones. The land topography comprises of the alpine highlands of about 3815 masl and also the lowland area with an altitude of 1600masl. Human populations in the rural and urban areas are estimated to be 88,100 and 59,591 respectively. The main occupation of rural population is mixed farming system whereby crop production and livestock production are managed hand- in- hand together. (Tiyo Wereda Agr. Data, 2003).

Poultry population in Tiyo Wereda is estimated to be 40648 out of which 2500 are exotic breeds of Rhode Island Red and Bovans breed. The indigenous local strain chickens found in the study area are not well studied and characterized for their genetic identity. However, the genetic potential of these chickens in egg and meat production is considered as low (Nasser, 1998). At the smallholder farmers level local strain chicken are reared in a traditional backyard system. The birds feed by scavenging around the residence area and occasionally supplemented with food residues and cereals. The housing and nesting place provision are poorly and constructed from the locally available materials (Tiyo Wereda Agr. Data, 2003). The main source of Rhode Island Red breed is Adama Poultry Breeding and Multiplication Center that they were sold to the farmers with certain subsidy through the Wereda Agricultural extension program. The Wereda Agricultural experts regularly supervise these birds for their productivity at the farmer's level and the farmers are not allowed to sale or change the originally distributed parents by any means.

Figure 3: The map of Ethiopia showing administrative boundaries of Zones.

Source: Ministry of Agriculture



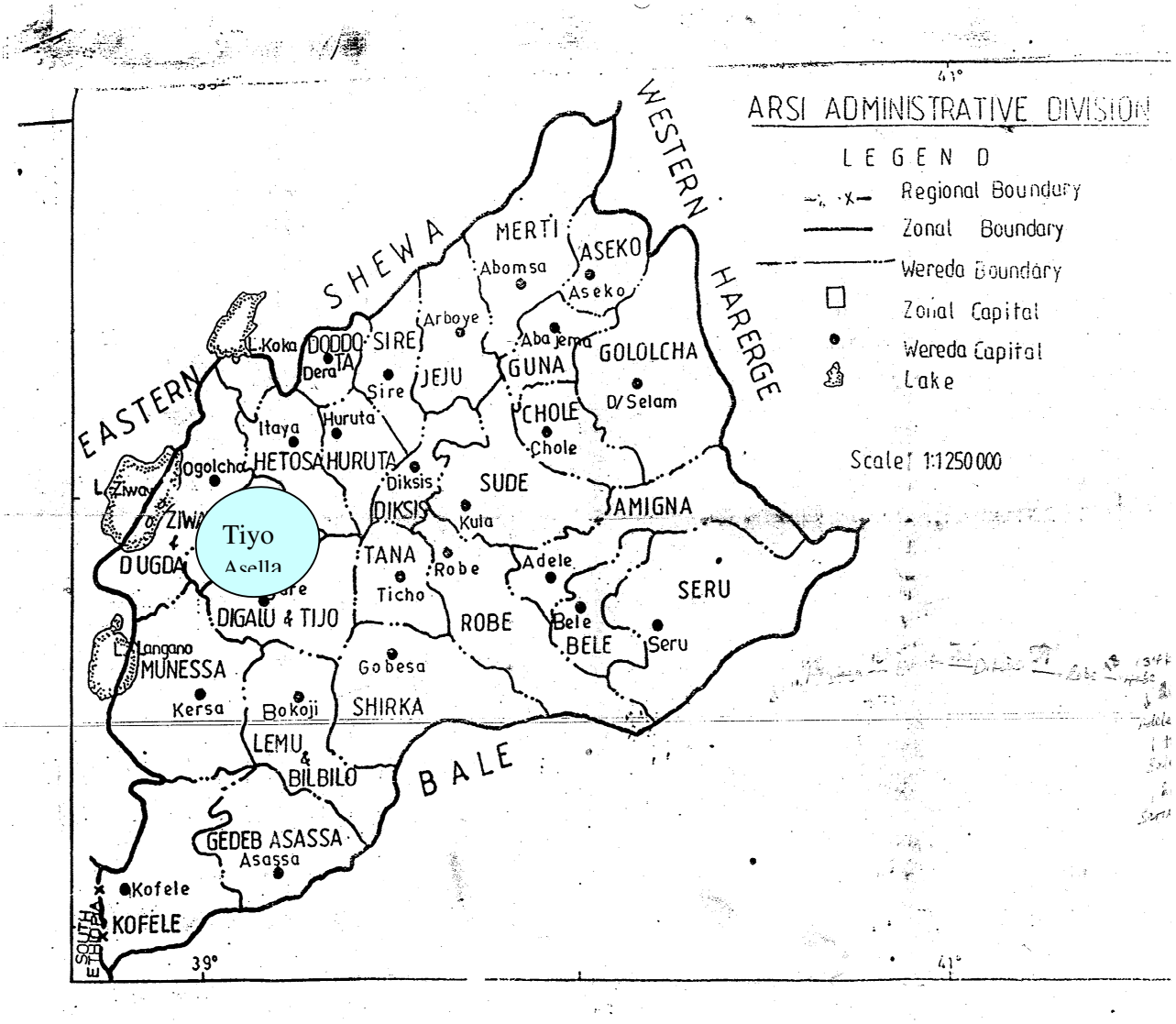


Figure 4: Arsi Administrative Zone, Wereda boundaries and the Shaded area is Tiyo Wereda

Source: Arsi Zone Planning and Economic Development Department

7.2. Study Population

Local strain chickens were bought directly from the farmers randomly in five market centers. Tiyo Wereda has five market centers in which chickens are available for sale and purchase. The names of these markets are Asella town, Beritti, Golja, Boru-jawe and Gondie. Both sexes were included proportionally. Physical appearance, size of the chicken and short interview of owners about the pedigree of their chicken was made to exclude exotic blood or

crossbreed chicken. Most chicken bought for the sample were approximately in the range of growers and adult age groups. The sampling method was systematic random sampling method and sample birds were purchased every 2 or 3 chicken's interval. However, in some markets, the numbers of chicken in the market were low to apply the above random sampling and we were forced to buy chickens as much as available to us..

The study was planned to investigate Rhode Island Red breed chicken that were kept under smallholder poultry production system. However, in practical reality these chickens were crossbreed with the local chickens since they were managed together under extensive system. Thus, it was difficult to get the pure breed except F_2 and F_3 generation. Therefore, the samples from Rhode Island Red breed were collected from Adama Poultry Breeding and Multiplication Center to observe the importance of coccidiosis in this breed and to compare the prevalence between breeds and management systems. The farm holds about 13000 chickens under intensive deep litter management and a total of 31 live birds with different age groups and equal sex proportion were randomly collected for the sample (Methusala *et al.,* 2002).

Sample size determination was based on the assumption of the possible prevalence rate of the disease recorded in other places and 15% expected prevalence rate was considered from previous researchers. The formula applied to calculate sample size was the formula for simple random sampling method and the study considered 95% level of significance (Thrusfield, 1996).

$$N = \frac{1.96^2 * p_{exp} * q}{d^2} \qquad N = 191$$

N= the required sample size

P_{exp}= Expected prevalence

$q = (1 - p_{exp})$

d = Desired absolute precision

7.3. Study Design

The study design consists of cross sectional study to determine the prevalence of coccidiosis in local strain and RIR breed chicken and to identify the prevalent coccidial species in the study area. Experimental study was conducted to compare the level of natural resistance

between local strain and Rhode Island Red (RIR) chickens by artificial infection with *Eimeria tenella* at Asella Regional Veterinary Laboratory. Questionnaire survey was also conducted to collect information from farmers and Adama poultry farm regarding the general condition about poultry production and disease problems in the study area.

7.4. Cross Sectional Study

The study was conducted from September -December 2003 GC. The study methodology involved quantitative and qualitative analysis of fecal examination to investigate oocyst discharge, necropsy examination to grade the intensity of lesions induced (lesion scoring), location of lesion in the intestine, identification of the species and histopathological examination were performed. Measurement the size and shape of sporulated oocyst was done to identify the species of coccidia observed in the study area.

7.4.1. Questionnaire Survey

The survey was conducted on 100 farmers having direct practice in poultry production from 10 Peasant Associations (PA), 10 farmers in each PA. Pre-tested questionnaire was developed and used to gather information regarding general production system in the study area, assessment of chicken disease occurrence and farmer's treatment practices. Disease assessment in PA's was based on information regarding clinical manifestations of some common diseases encountered and their seasonal occurrence.

7.4.2. Fecal Examination and *Eimeria* Species Identification

The local strain chicken purchased was kept overnight in the laboratory for at least 18 hours. The aim was to collect fecal sample from each individual bird and to record ante mortem clinical condition of each bird. The fecal sample of each bird was blended by mortar and pistol and oocyst per gram feces (OPG) was calculated based on the technique described by MAFF, (1979) (Annex 3). Positive samples were further examined for species identification based on oocyst size, sporulation time, shape and color of the oocysts.

The procedure applied to harvest oocysts was by floatation technique using saturated salt solution (Conway and McKenzie, 1991). The oocysts recovered through floatation method were put on the microscope slide, examined under 40x objective and 10x ocular micrometer eyepiece using the calibrated microscope. The average length and width were measured from at least 10 oocysts to determine the size. Oocyst shape and color was also recorded for each recovered positive cases. Calibration of the objective lens was done based on the procedures 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 and centrifuged 3-4 times. The salt-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, 1991).The technique for oocyst sporulation is described in (Annex 4). The suspension was examined by hemocytometer chamber every 3 hours during the working hours to determine the sporulation time and sporulation time was considered when 90% of the oocysts were sporulated. After the sporulation time is determined the oocysts were preserved in 2.5% potassium dichromate and stored at 4^{0}C. Oocyst color and size is mostly helpful characteristics for identification of *E. maxima* that is brown red color and significantly large in size.

7.4.3. Gross Lesion Examination

During 18-24 hours stay of chicken in the laboratory ante mortem clinical condition of each bird was recorded. The chicken was euthenized by cervical dislocation using the technique described by Zander, (1976). The gastrointestinal tract was grossly examined carefully. 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. Intestinal gross lesions in any part of the sections were graded from 0 to 4 based on lesion score key (Conway and McKenzie, 1991). The lesion score zero represents absence of lesion and lesion score four is for very severe intestinal /cecal mucosa lesion and fatal cases. The location of the lesion

was recorded; intestinal contents from the respective sections were taken and duplicate mucosal scrapping smears made from each section of the intestine.

7.4.4. Histopathological examination:

Classical lesions were taken for histopathological preparation. Haematoxylin and eosin staining was used to demonstrate the developmental stages in the cecum (MAFF, 1979). Tissues sampled was fixed in 10% neutral buffered formalin, sectioned at 5-6µm thicknesses and stained with haematoxylin- eosin stain (Luna, 1968).

7.5. Experimental Study

7.5.1. *E. tenella* Stock Preparation and Oocyst Dosage

Donor chickens of about 3 months of age were obtained from Debre Zeit Agricultural Research Center. They were checked for coccidial infection prior to artificial inoculation and were found to have no coccidial infection. The sporulated field isolate *E. tenella,* recovered during the cross sectional study, was given at the dose rate of 3850-4000 oocysts per bird (Williams, 2001). One ml of oocyst suspension in distilled water was orally inoculated directly in to the crop using a flexible plastic tube fitted to 5ml syringe. Fecal examination for oocyst was started on day 4 and the droppings were collected twice in a day. The birds started shading oocysts 128 hours post infection.

7.5.2. Experimental Birds and Housing

Eggs of local strain chicken were purchased from markets that have been selected for the cross sectional study in Tiyo Wereda. Relevant information regarding origin, pedigree, fertility (presence of cock) was obtained. Eggs were also checked for shape, size, shell surface smoothness and physical damages before purchase. They were stored in a relatively cold temperature for short time until they were incubated in to the incubator. Rhode Island Red breed eggs were purchased from Adama Poultry Multiplication and Breeding Center. The

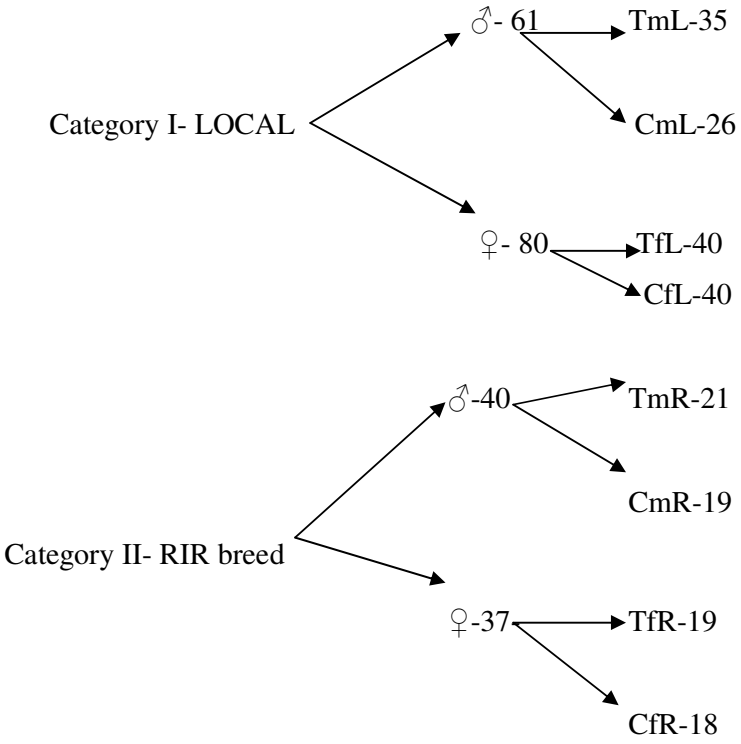
eggs from both genotype chickens were incubated in the same date at Debre Zeit Agricultural Research Center to obtain same age group under the same management condition. The number of eggs purchased, incubated and hatched in local strain chicken was 479, 396 and 187 respectively and in the RIR breed were 350, 350 and 80 respectively.

A total of 267 chicks were immediately transported to Asella soon after the hatching and were kept under coccidia free conditions for 30 days. They were reared on deep litter consisting of sawdust (3kg/ m^2) and mixed with limestone (0.5kg /m^2) to prevent moisture and four rooms were used for the experiment. Commercially available starter ration of crude protein (CP) 21.0%, 2833 kcal metabolizable energy per kg diet and optimum vitamins and trace minerals were included and fed up to day 30. No anti-coccidial drugs were given. Heat supply to keep the chicks warm was by a hover fitted with 200 and 150 watt electric bulbs and the amount of heat in the room was gradually decreased and maintained at 21^0C during the experimental work. The lighting regimen was 24 hours light cycle (Graat *et al.,* 1996).

7.5.3. Experimental Groups

On day 30, the body weight and packed cell volume (PCV) of all chicks were taken and categorized into different experimental groups by random allocation. Underweight and weak chicks were excluded from the experiment groups. In both genetic groups, a total of 218 chicks were assigned randomly in to four test and four control groups. The sex ratio within groups was proportional (Figure 5). N = 218 chicks

Figure 5: Experimental group random allocation in local strains and RIR breed



TmL = Local male test group

TfL= Local female test group

CmL= Local male control group

CfL= Local female control group

TmR= RIR male test group

TfR= RIR female test group

CmR= RIR male control group

CfR= RIR female control group

Fecal samples from all experimental groups were collected and examined for any contamination by coccidia parasites prior to the experimental infection. All groups were found negative for coccidial oocysts. On day 31, each test group was inoculated by $1.5x10^5$ sporulated oocysts of *E. tenella* into the crop in 1ml of distilled water suspension. All experimental groups comprised two replicate cages with random allocation to each cage. The uninfected control groups in both genotype chicken and sexes were sham inoculated with distilled water. Water intake was restricted only for one day after inoculation. Feed and water was then provided ad libtum.

Fecal examination and physical clinical sign observation started on day 5 post infection. Clinical signs of the test groups were recorded twice in a day. The fecal droppings in each

cage were collected on a polyethylene sheet placed on the fecal tray of the cages. The total oocyst production of 24 hour pooled fecal samples from each cage were calculated for each chick and recorded up to day 7 of post infection. Diarrhea and fecal blood sign, the number of sick and dead chicks were recorded twice in a day until day 7 post infection. On day 8 post infection, all chicks in all experimental groups were weighed and PCV measures were recorded for the second time. On the same date, the birds were scarified for cecal lesion score based on the procedures described by Conway and McKenzie, (1991). A score of zero is normal and a score of four is assigned for the most severe and dead birds due to coccidial infection during the patent period.

7.5.4. The Experimental Parameters

7.5.4.1. Body weight gain/ loss (Bwtd):

Body weight on day 30 before experimental infection was designated as Bwtb and on day 8 of post infection as Bwta. At day 8 post infection, all birds were slaughtered. Body weight gain/ loss (Bwtd) between day 30 and 38 was calculated for each chick (Bwta –Bwtb) for birds surviving the experimental infection (Pinard-Van Der Laan *et al.,* 1998).

7.5.4.2. Packed Cell Volume (PCV) value:

Packed cell volume value was measured twice; prior to infection on day 30 (PCVb) and on day 38 (PCVa) before necropsy examination. The reduction in packed cell volume (PCVr) of the test and control groups was compared to illustrate the change using the following formula.

Reduction in PCV (PCVr) = 100* (1-PCVa/PCVb) (Lillehoj, *et al*., 1989)

7.5.4.3. Fecal oocyst output:

Fecal sample collection was started on day 5 post infection and the droppings were pooled and collected once every 24 hours from individual cages. Then the total oocyst production per day was calculated using the McMaster egg/ oocyst counting technique (Annex 5) using the following formula (MAFF, 1979).

Total oocyst production (TOP) per bird in 24 hours sample was:

$$TOP = \frac{X}{0.15} * vol. * 10$$

Where X= number of oocysts counted

Vol. = 1500ml water that the feces is soaked

0.15= volume of the McMaster counting chamber

10= correction factor

The number of oocysts recorded from each cage in three consecutive patent periods was divided by the number of chicks in that cage and expressed as the average number of oocyst produced per bird (MAFF, 1979).

7.5.4.4. Morbidity rate

Gross examination for traces of blood and diarrhea on the fecal tray and changes in the health condition was considered as a sign of morbidity. Clinical manifestation of the disease such as dropping wings, depression, decrease feed intake, huddle together as if they feel cold in each cage were observed and the number of chicks prominently showing the signs were recorded to calculate the morbidity rate. Morbidity rate was calculated from the number of chicks that appeared sick during experimental period divided by the average number of chicks in that experimental group for 3 consecutive days of patent period. Dead chicks were weighed, necropsy examination was conducted and the cause of death was determined.

$$\text{Morbidity Rate} = \frac{\text{Total numbers of sick chicks}}{\text{The average numbers of at risk chicks in that experimental group} * (3 \text{ days})}$$

7.5.4.5. Lesion scores**:**

Cecal lesion score was graded according to the guidelines described by Conway and McKenzie, (1991). Impression smears were also taken from some lesions and gram staining was made to microscopically observe microbial complications. Cecal mucosa scrapping was also taken and observed by direct smear to observe different developmental stages of *E. tenella* microscopically.

7.6. Statistical Analysis

The association of oocyst count per gram feces (OPG) between the two genotype chicken and sex was computed by chi-square and Kruskal Wallis tests for the cross sectional study. The mean OPG count, prevalence rate and confidence interval in both genotype chickens were calculated using Stata-7. XP- Professionals 2001 Mc Excel Window facilitated the graphs and summary tabulations of prevalence rate and frequency occurrence of coccidial species. The p-value for statistically analyzed data was considered significant different at 5% probability level (Methusala *et al*., 2002).

The randomized block design model was applied for the comparison of the two genotype chickens. Comparison between the two genotype chickens and sex effect was analyzed by two- way analysis of variance with interaction, and student's t-test was used to separate significantly different means using statistical package Stata 7. Morbidity rate analysis between breed and sex was undertaken by logistic regression model (Pinard- Van Der Laan *et al.,* 1998). Treatment differences were considered significant at the 0.05% probability level.

The model was: $y_{ijk} = \mu + A_i + B_j + e_{ijk}$

Where y_{ijk} is the k^{th} observation in the i^{th} block for the j^{th} treatment. μ is the overall mean, A_i is a fixed effect of the i^{th} block (i = 1and 2), B_j is a fixed effect of the j^{th} treatment (j = 1, 2, 3 and 4) and e_{ijk} is the error term.

8. RESULTS

8.1. Cross sectional Study

8.1.1. Questionnaire Survey

Poultry management in the rural area is free ranging and chicken feed by scavenging around the house with occasional cereal and food residuals supplement. Poultry production and income generated from poultry production in the rural community is the major income source for females and children in greater than 75% respondents. Disease problems in traditionally managed chicken are very important and more than 90% interviewees responded as medium to high rank for disease problems. Although farmers have their own local names and ways of identifying poultry diseases, the most frequent disease they complain about (>75%) was diarrhea. The other interesting observation was the seasonal occurrence of diarrhea and more than 85% farmers have clearly described the color of diarrhea with the season for frequent occurrence. Therefore, most respondent (>75%) described that bloody diarrhea predominantly appeared during wet season than chalky, yellow or green diarrhea. This observation might be more indicative of the occurrence of coccidiosis. Moreover, the public and private veterinary service centers have no anti coccidial drugs and other medicaments used for poultry diseases. More than 80% farmers have experience of brooding and rearing chicks during dry season.

8.1.2. Potential risk factors for coccidiosis

In free-ranging local chickens, non-selective picking behavior during feeding can expose chickens to infection. Age group and high moisture conditions that favorably influence oocyst sporulation and development to the infective stage were the potential risk factors. However, in Rhode Island Red breeds that were kept under intensive deep litter system, the potential risks observed from farm assessment through questionnaire were age groups, production systems, flock size, moisture level in the poultry house and level of biosecurity.

8.1.3. Clinical Coccidiosis finding

In the cross sectional study, chicken that showed depression, ruffled feather, diarrhea and/or blood mixed droppings were recorded as clinical cases. The results of clinical and sub clinical infection are shown in Table 2.The number of clinical cases in RIR breed and local strain chickens was not significantly different. Clinical coccidiosis detection between male and female was also not significantly different in both genotype chickens Table 3.

Table 2: Clinical and sub-clinical coccidial infection cases

No	Breed	Samples examined	Clinical	Sub-clinical	Total positive cases	(%) of clinical	(%) of positive cases	95%C I clinical (%)
1	Local	160	20	78	98	12.5[a]	61.25[a]	7.3-17.7
2	RIR	31	7	18	25	22.58[a]	80.65[b]	7.0-38.2
	Total	191	27	96	123	14.14	70.95	

Mean values within a column followed by different lower case superscript are significantly different ($p<0.05$).

8.1.4. Quantitative and Qualitative Fecal Examination

The prevalence of coccidial infection and OPG count in local strain and Rhode Island Red chicken is shown on Table 3. The frequency of occurrence of the coccidial infection in RIR breed was significantly higher than the local strains ($Chi2 = 4.26$; $p <0.05$) (Figure 6). The OPG count in RIR breed was also significantly higher than the local strain chicken ($Chi2 = 109.085$; $p < 0.001$). However, the frequency occurrence of coccidial infection between male and female was not statistically significant. Moreover, OPG count of male and female was not significantly different. The association of clinical case occurrence with OPG counts was highly significant in both genotype chickens ($Chi2 = 106.82$; $p < 0.001$).

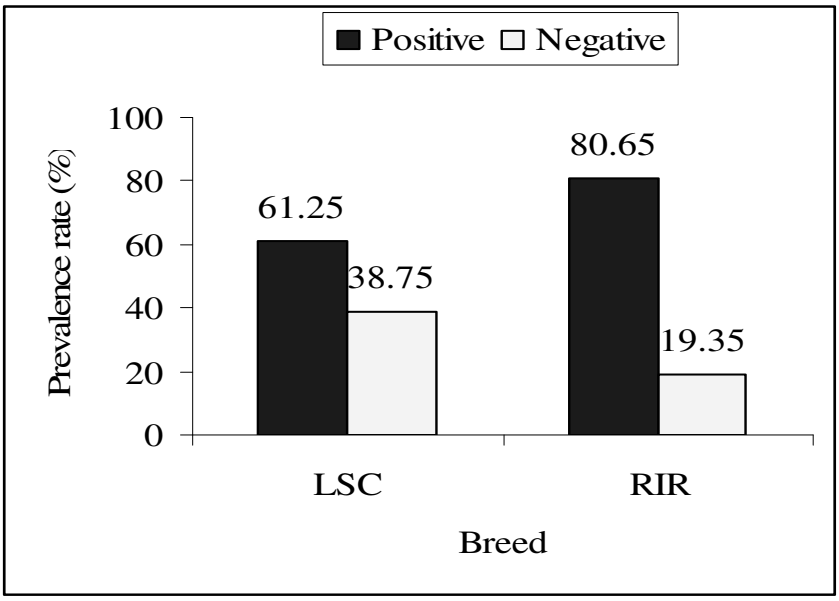


Figure 6: Frequency occurrence of coccidial infections in Local strain and Rhode Island Red chicken

Table 3: Frequency detection of fecal oocysts in local strain chickens and RIR breed.

No	Division	Category	Manag system	Samp. examined	Positive samp	Clinical cases	Preva rate%	C I of preva. rate%	Mean OPG± SE	C I of OPG
1	Breed	LSC	F-r	160	98	20	61.25[a]	53.62 - 68.88	1809± 251[a]	1314 - 2304
		RIR	D-l	31	25	7	80.65[b]	65.91 - 95.38	5215± 106[b]	3161 - 7271
2	Sex	Male	-	90	59	15	65.56[c]	55.54 - 75.56	2017± 99[c]	1423 - 2610
		Female	-	101	64	12	63.37[c]	53.81 - 72.93	2669± 457[c]	1763 - 3575

Mean values within a column followed by different lower case superscript (a-d) are significantly different ($P < 0.05$).

Manag- Management F-r = Free range

Samp- Sample D-l = Deep litter

Preva- Prevalence

Coccidial species identified in this study were *E. tenella, E. acervulina, E. necatrix, E. maxima, and E. mitis* in descending order of their occurrence (Table 4). Mixed infections were the predominant case, which accounts for 45 mixed infections. The major proportion of mixed infections amounting to more than 55.6% was consisted of *E acervulina* as a member of the mixed infection. *E. necatrix* with *E. acervulina* and *E. maxima* with *E. acervulina* have occurred in the highest mixed infection frequency. *E. mitis* was the first tentatively diagnosed species of coccidia in this study predominantly from the samples collected from Adama poultry center. It was the smallest in size as compared to others and sub spherical shape. The size and color of most coccidial species are overlapping to identify based on their morphological feature except for *E. maxima* which has the largest size and brown red color (Figure 7). The average length and width of each species and their identification characteristics are shown in (Table 4). Most coccidial species were restricted to specific predilection sites in the intestine. However, *E. acervulina* and *E. maxima* were found to occupy the most proportion of the small intestine than other species.

Oocyst sporulation time for *E. acervulina* and *E. mitis* were shorter (24-28 hours) as compared to other species. *E. tenella* and *E. necatrix* had similar sporulation time of about 36 hours and oocysts of *E. maxima* took almost 48 hours for sporulation at room temperature (Table 4).

Table 4: Coccidial species investigated in the study area and their identification characteristics.

No	Species identification characters	*E. tenella*	*E. necatrix*	*E acervulina*	*E. maxima*	*E mitis*
1	Av. Oocyst length(µm)	22.77	22	18.64	30.26	16.26
2	Av. Oocyst width(µm)	19.37	19.25	16.46	23.78	15.61
3	Shape index	1.18	1.143	1.132	1.27	1.042
4	Frequency occurrence	29	16	23	13	9
5	Oocyst shape	ovoidal	oblong ovoid	ovoid	ovoid	sub spherical
6	sporulation time (Hrs)	36	36	24-28	36-48	24-28
7	Site of occurrencein the intestine	cecum	mid intestine (Jejunum and ileum)	upper intestine (Duodenum)	mid intestine (Jejunum and ileum)	upper intestine (Duodenum Jejunum)
8	Pathological lesions induced in host intestine	Hemorrhage & bleeding thickening of cecal wall	Ballooning, Bleeding, white plaques &thickening of int. wall	White round colonies, streaks & mucoide exudates	Ballooning, petechial. hemorrhage, mucoid exudates & thickening of int. wall	no, apparent lesion

8.1.5. Gross Lesions

Post mortem examination is the best method for diagnosis and species identification. The site of their occurrence and characteristic lesions produced by specific *Eimeria* species were used for identification of the species (Table 4). *E. tenella* induced hemorrhage, thickening of the mucosa, clotted blood and cecal cores in the cecum depending on the magnitude of infection and duration of the infection (Figure 8, 9). In some cases concurrent infections with bacterial enteritis obscured the coccidial lesions in duodenum and cecum. *E. necatrix* and *E. maxima* usually shared similar intestinal lesions. Lesions in *E. necatrix* were more severe with bleeding and whitish plaques seen in the middle intestine on both sides from yolk sac diverticulum's, which was not the case in *E. maxima* (Figure 10*). E. acervulina* usually occurred in the duodenal loop characterized by mucoid exudates in intestinal content; white spots were usually evident from the serosal side and eroded mucosal membranes.

The total number of chicken in which intestinal lesions were scored above zero was 98 (61%) in local strain and 25(81%) in RIR breed. The frequency detection of lesions in RIR was significantly higher than it appeared in local strain chickens ($p < 0.05$). The detection of intestinal lesions had significant association with the occurrence of clinical coccidiosis ($Chi2 = 17.38$; $P< 0.001$). Moreover, lesion score showed significant association with OPG counts in both genotype chicken ($Chi2 = 89.72$, $p < 0.01$). Lesion score between male and female was not significantly different. Microscopic examination of intestinal mucosa scrapping was also made in addition to gross lesion grading to observe different developmental stages of coccidia in the mucosa.

8.1.6. Histopathological examination

Histological sections prepared from classical lesions of *E. tenella* and *E. necatrix* were examined. Pathological lesions observed in the cecum section showed that necrotized epithelial cells denuded from the mucosal layer and severe hemorrhage in the lamina propria (Figure 11). The crypt cells were highly invaded with the developmental stages of *E. tenella* schizonts and gametocytes that their morphology is almost disappeared (Figure 12). The lesion in the middle intestine due to *E. necatrix* also consisted cryptal and absorptive

epithelial cell destruction and sub mucosal edema. The large size schizonts and gametocytes have occupied the large proportion of the crypt cells (Figure 14). Inflammatory cells infiltration into the lamina propria was observed (Figure 13). Heterophil cells predominantly infiltrates into the site when necrosis was intensive (Mesfin *et al*., 1978).

Figure 7: Arrow M shows Sporulated *E. maxima* oocyst (160x).

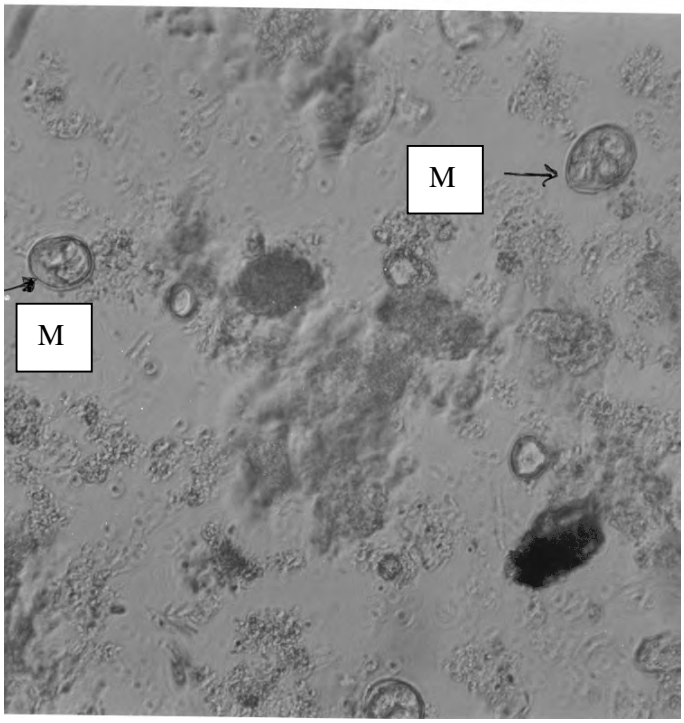
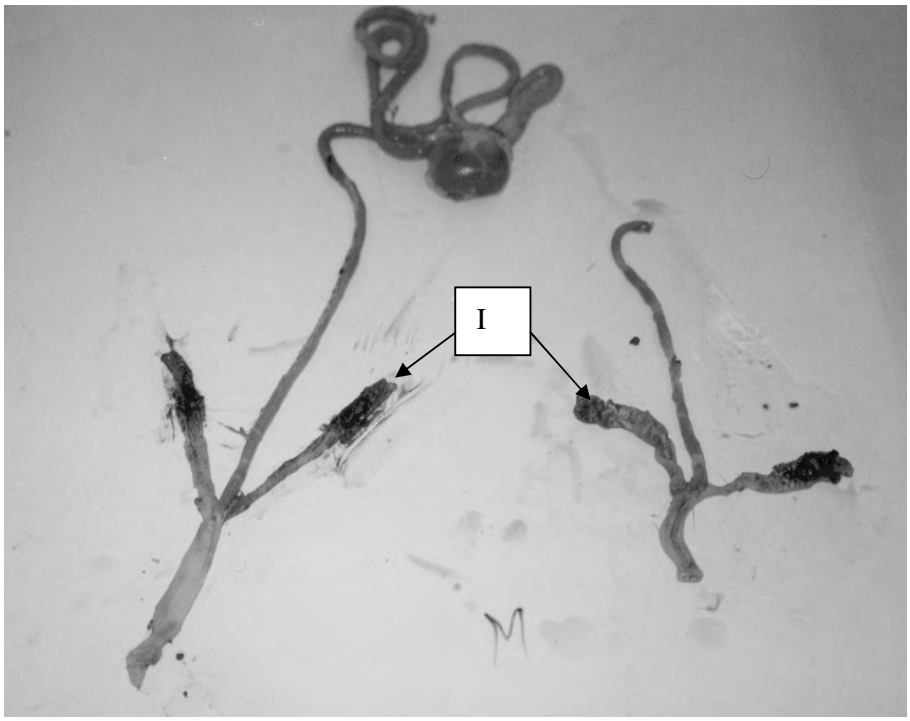
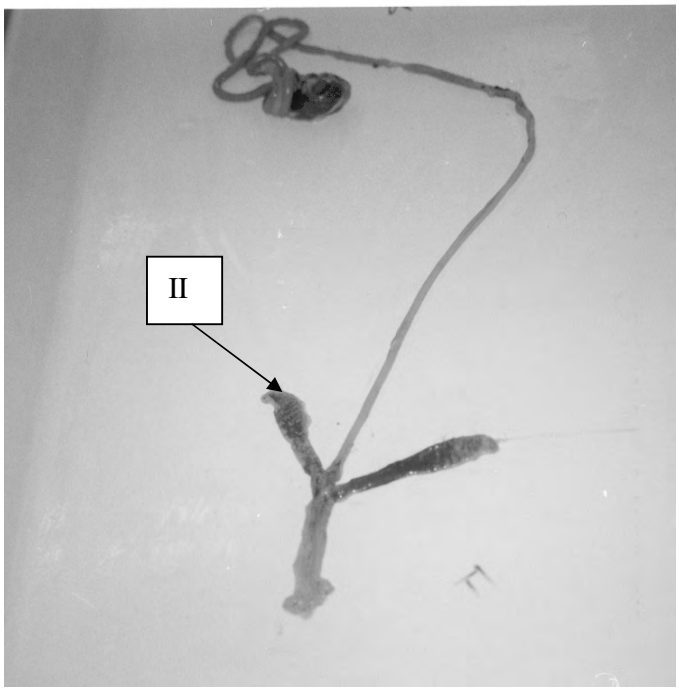


Figure 8: Lesion in the cecum caused by *E. tenella* graded as 4 lesion score.



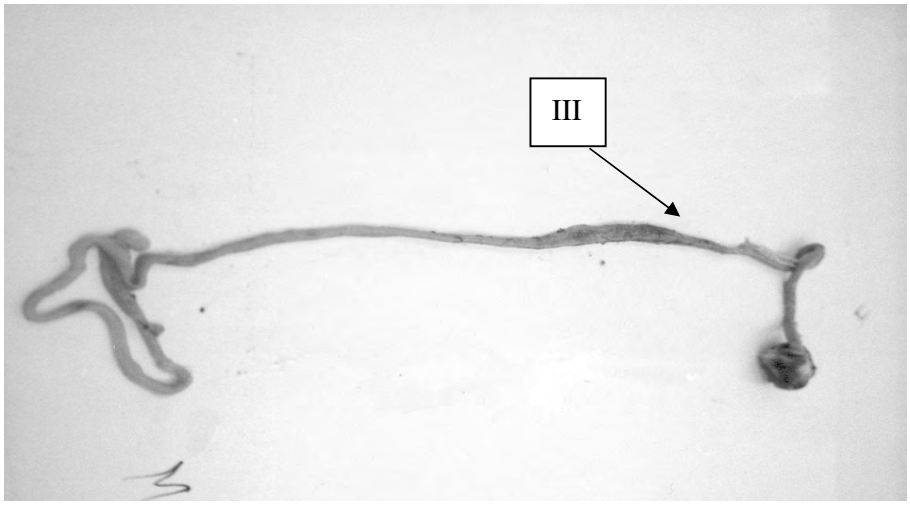
Arrow I show opened cecum filled with clotted blood and mucosal ulceration

Figure 9: Lesion in the cecum caused by *E. tenella* graded as 3 lesion score.



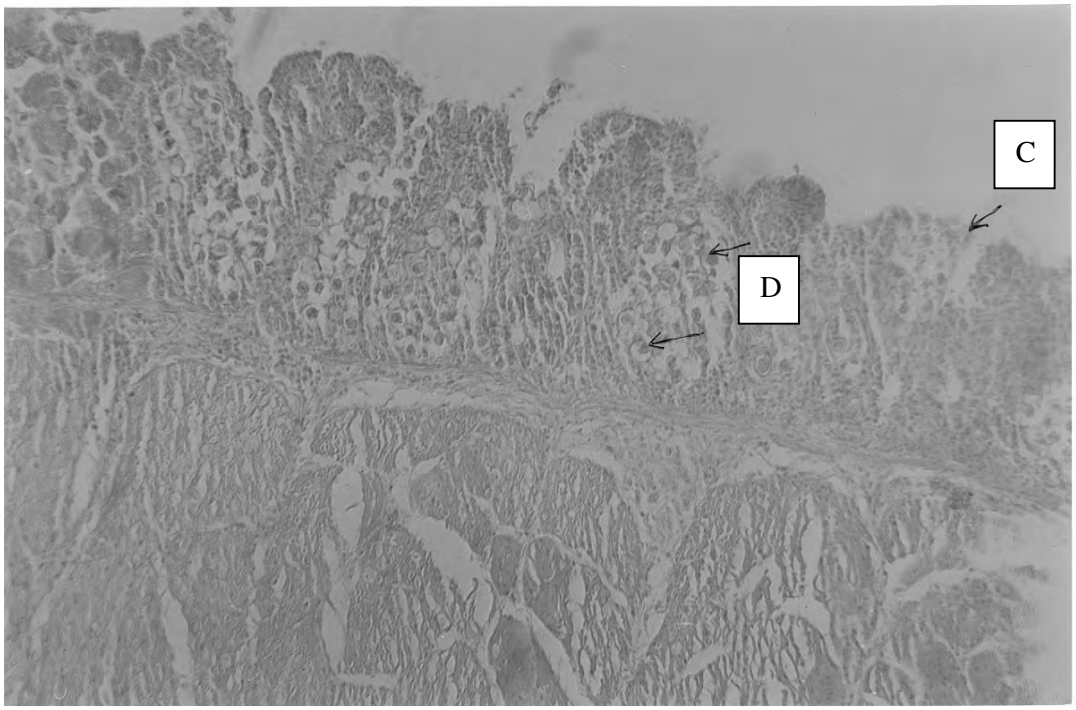
II- Opened cecum with hemorrhage and eroded mucosal layer

Figure 10: Lesion in the middle intestine caused by *E. necatrix* graded as lesion score 3.



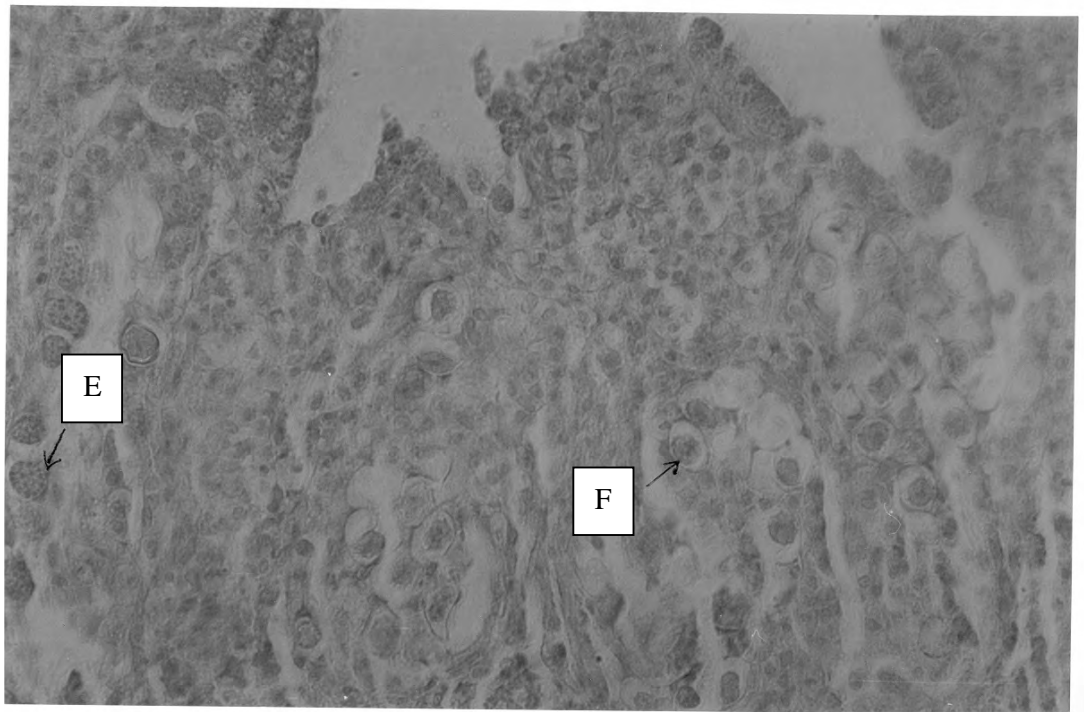
III Opened intestine, thickening of the intestinal wall, with hemorrhage and bleeding and necrotic lesion in the mucosa.

Figure 11: Cecum section infected with *E. tenella* (160x).



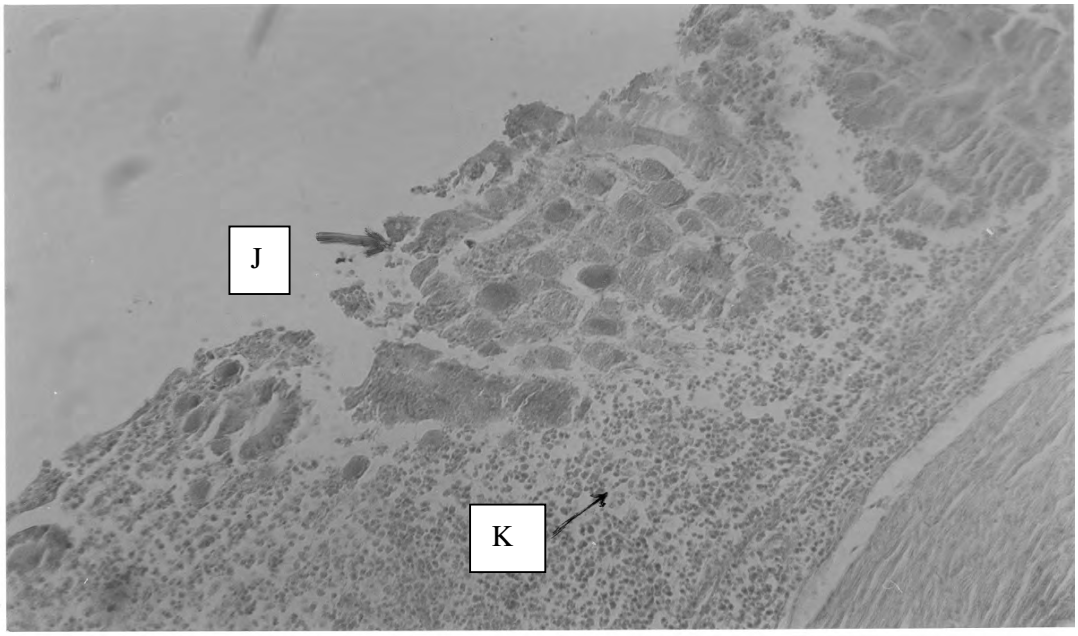
Arrow C denuded epithelial layer. Arrow D shows the crypt cells are invaded by developmental stage schizonts

Figure 12: Cecum section infected with *E. tenella* (400x).



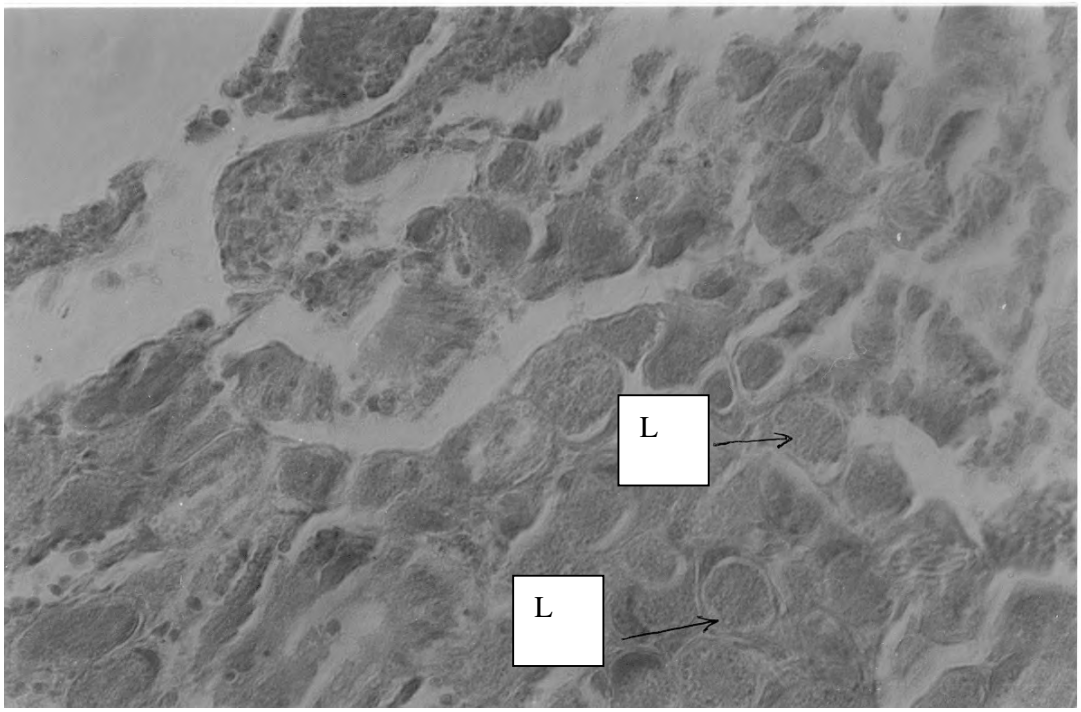
Arrow E and F show gametocytes and developing schizonts of *E. tenella*.

Figure 13: Ileum section infected with *E. necatrix* (160x).



Arrow J shows necrotized and denuded epithelial mucosa. Arrow K high inflammatory cells influxes into the sub mucosa and thickened sub mucosal layer.

Figure 14: Ileum section infected with *E. necatrix* (400x).



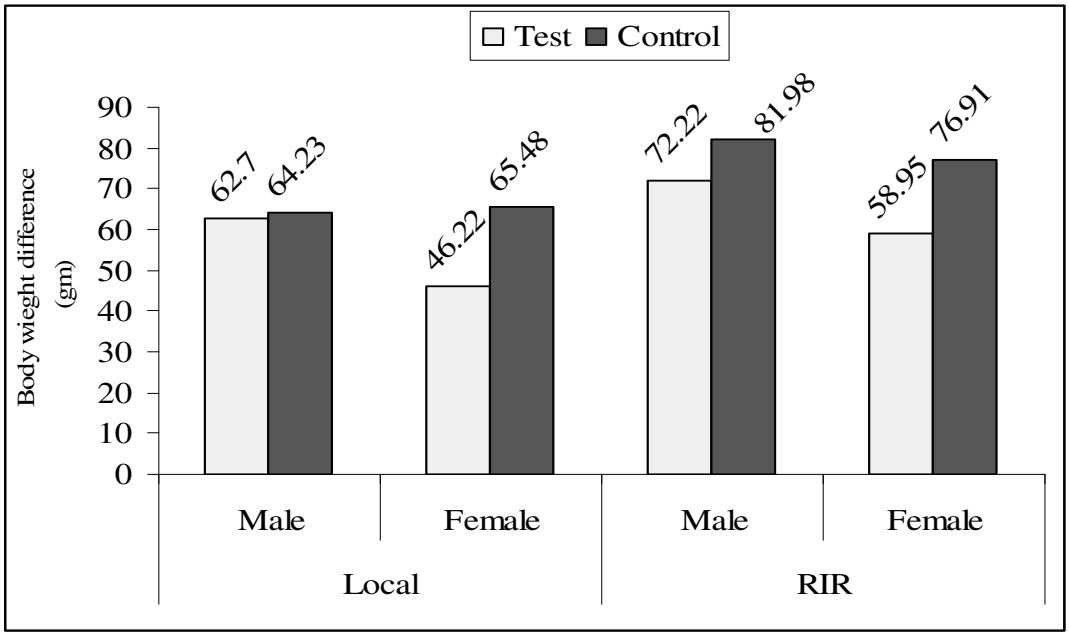
Arrow L shows mature and immature large schizonts of *E. necatrix*.

8.2. Experimental Outcome Measurements

8.2.1. Body weight difference

The mean body weight difference in genotype chickens and sexes were compared between the test and control experimental groups (Annex 1). Body weight difference comparison between test and control groups of local strain chickens showed there was significant body weight depression in the test groups ($p < 0.01$). However, body weight difference between test and control groups of RIR showed that the body weight depression in the test groups was significant at ($p < 0.05$) (Figure 15). The body weight difference of females between the test and control groups in both local strain and RIR breed were significantly different ($p < 0.01$ and $P < 0.05$ respectively). However, body weight difference of males in both RIR and local strain were not significantly different between test and control groups.

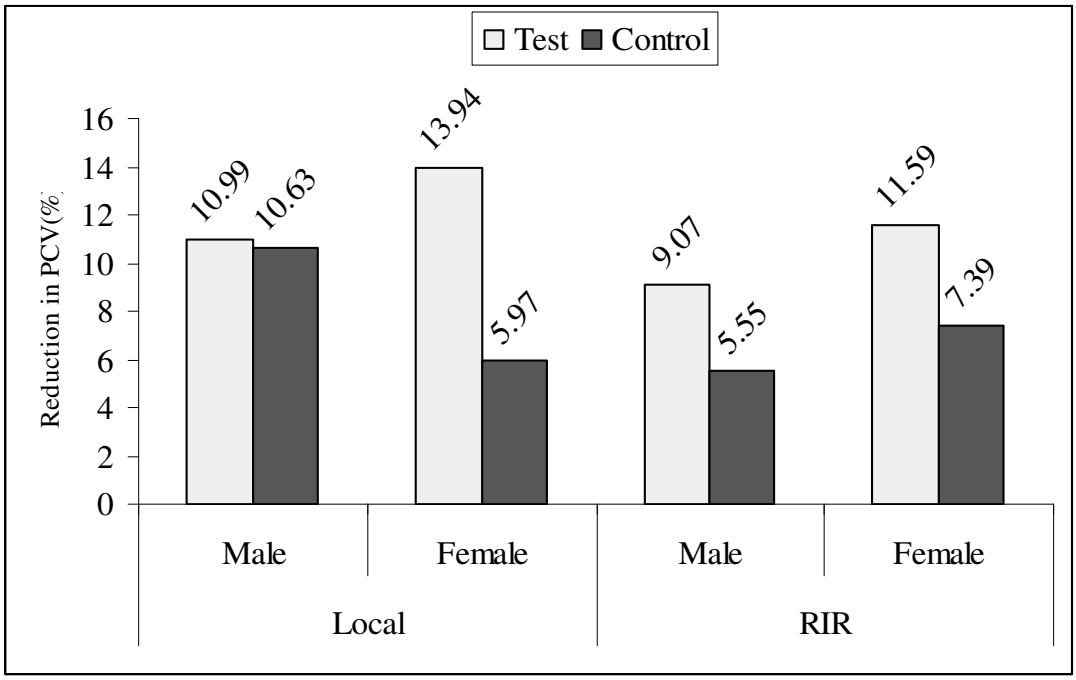
Figure 15: Mean body weight gain/ loss of the experimental groups.



8.2.2. Reduction in Packed Cell Volume (PCVr %) :

The effect of coccidiosis on PCV values was analyzed based on the calculated PCV reduction (PCVr) values between the test and control groups for each experimental group. The packed cell volume before inoculation (PCVb) of both genotype chickens in all experimental groups was not significantly different. However, the reduction in PCVr between the test and control groups of local strain and RIR chicken were significantly different ($p < 0.01$and $p < 0.01$ respectively) (Figure 16). The PCVr between the test and control groups of local strain female birds was significantly different ($p <0.001$). Moreover, PCVr between the test and control groups of RIR breed in both sexes were significantly different ($p <0.05$ and $p <0.05$ respectively). However, the PCVr value between the test and control groups of local strain male was not significantly different.

Figure 16: Packed cell volume reduction (PCVr) by genotype Chicken and sex.



8.2.3. Oocyst Production

Oocyst shading commenced on day 5 post infection in most experimental groups. All control groups in both genotypes and sexes showed no oocyst production in their droppings throughout the experimental period (Table 5). RIR breed shaded significantly higher oocysts than the local strain chicken ($p < 0.01$). Comparison of sexes showed test group females shaded significantly larger oocysts than males in RIR breed ($p < 0.05$). However, there was no significant difference in total oocyst production between male and female birds of local strain chickens. Total oocyst production has a positive correlation with lesion score at $p < 0.01$ level of significance ($r = 0.661$) Annex 2.

8.2.4. Morbidity and Mortality

Noticeable signs of morbidity were observed on day 5 post infection and peaked on day 6 and 7 with fecal blood signs. The morbidity rate between the two genotype chicken showed that

local strains had significantly higher ($p < 0.01$) morbidity rates than RIR breed (Table 5). However, the morbidity rate between males and females were not significant in both local strain and RIR chickens. No morbidity was recorded in all control group birds. Two birds died on day 5 and 6 post infection, one male and one female each from local strain chicken. The dead chicks were subjected to post mortem examination and the lesions were localized to the cecum. The cecal pouches were filled with clotted blood, distended, thickened and ecchymotic hemorrhages on the mucosa wall.

8.2.5. Lesion Scores

The mean lesion score in the test groups of RIR breed were significantly higher than the local strain chickens ($p \leq 0.001$). However, there was no difference between males and females with respect to lesion score in both genotypes (Table 5). Lesion scores had a positive correlation with total oocyst production and morbidity rate at ($p < 0.01$) level of significance ($r = 0.661$ and 0.771 respectively) (Annex 2). No lesion was observed in the control groups of both genetic groups.

Table 5: Mean body weight difference, PCV (Packed Cell Volume) values, total oocyst production, lesion score and morbidity rate by breed and sex.

Breed	Sex	Exper. group	No. birds	Bwtd in (gm)	PCVa (%)	PCVr (%)	TOP in (10^4)	Lesion score	Morbidity
LSC	M	Test	35	62.7^{c}	35.02^{a}	10.99^{a}	1779^{a}	2.63^{a}	0.222^{a}
		Control	26	64.23^{c}	38.96^{b}	10.63^{a}	0	0	0
	F	Test	40	46.22^{a}	33^{c}	13.94^{b}	1913^{a}	2.625^{a}	0.236^{a}
		Control	40	65.48^{b}	39.45^{d}	5.97^{c}	0	0	0
RIR	M	Test	21	72.22^{ab}	34.98^{ab}	9.07^{ab}	2172^{c}	3.286^{b}	0.111^{c}
		Control	19	81.98^{ab}	39.21^{ac}	5.55^{ac}	0	0	0
	F	Test	19	58.95^{c}	33.74^{bc}	11.6^{bc}	2616^{d}	3.42^{b}	0.123^{c}
		Control	18	76.91^{d}	38.97^{bd}	7.39^{bd}	0	0	0

Mean values within a column followed by different lower case superscript (a-d) are significantly different ($p < 0.05$) between experimental groups.

M – Male, F – Female

9. DISCUSSION

9.1. Disease Prevalence and Species Identification

Generally, in traditional poultry production system, the input required is minimal and is considered as secondary to other agricultural activities by the smallholder farmers. Housewives and children are usually responsible to undertake poultry production around the homestead. Since these social groups usually stay longer around the home, they can easily look after the chicken. The income generated from poultry production may also be most accessible source of income during need of cash for women and children. Thus, from this point of view, poultry production may address the social and economical problems of gender issues and improve the income source and long-term economic potential of women in the rural community.

Indigenous knowledge of farmers on poultry coccidiosis might not be very specific. Nevertheless, they have keen awareness regarding the risk factors for the occurrence of the disease and they describe the disease based on the clinical signs. The current observation showed that public and private veterinary services overlook the health impact on poultry production and no drugs and biological preparations for poultry were available in their stock. Therefore, farmers resort to their own indigenous practices to treat and control chicken diseases, which may not be usually effective. The magnitude of the disease problem based on the farmer's response was highly amenable to appropriate technical interventions to increase the production and productivity of this resource.

The prevalence of coccidial infections in the cross sectional study in local strain chickens in this study was higher than in previous findings (Gual, 1990, Ashenafi, *et al*., 2001). The climatic conditions, agro-ecological set-up and lack of adequate information on the subject may be attributed to the variation. The relatively wet climate and cooler temperatures in the high and mid altitudes of the study area may be more favorable for the occurrence of coccidiosis. The findings consolidate the importance of coccidiosis in the indigenous chicken.

The frequency occurrence of coccidial infection in RIR breed was significantly higher ($p < 0.05$) than the local strain and this could be due to management system and breed factor. The higher OPG count in RIR breed can be related with the higher frequency occurrence of coccidiosis in the deep litter system due to relatively higher oocyst accumulation in the deep litter. The amount of oocyst discharged from infected chicken depends on the dose of oocysts ingested and the immunological status acquired from pre-exposure (Bumstead *et al*., 1989; Williams, 2001). Thus, the opportunity of the chickens to pick-up large numbers of sporulated oocyst can be more likely in the RIR kept in deep litter management than the local strain chickens. This result is consistent with the finding in large and small-scale deep litter rearing systems (Methusela *et al*., 2002). However, coccidiosis occurrence was not significantly affected by sex. This indicates there is no significant natural resistance variation in relation to the sex (Pinard- Van Der Laan *et al.,* 1998). The number of sub clinical coccidial infections was significantly higher than the clinical cases in both management systems. The birds might have developed immunity to the trickle infections acquired from the environment and maintain the state of balance by developing sub clinical disease. However, the morbidity losses may be considerably expensive without the producer being aware that his flock has any disease problem (Gordon and Jordan, 1982).

Previous researchers have reported the most important species of coccidia *E. tenella, E. acervulina, E. necatrix* and *E. maxima* in Ethiopia. Nevertheless, *E. mitis* was tentatively diagnosed based on morphological and other characteristics from the samples collected from Adama Poultry Center. Further confirmation of the identity of this species may be required in referral laboratory. The distribution of coccidial species in free-ranging chicken and under intensive management was not significantly different. However, *E. acervulina* was the most dominant species occurring in the intensive deep litter system, which is in agreement with the findings of Methusela, *et al., (*2002). The higher biotic potential of this species may favor its dominance occurrence in a confined production system. Due to this fact Reid, (1978) has stated that the significance of this species as a pathogen has increased steadily within the recent years in large poultry establishments. Mixed infections were frequently encountered, which account for 45 mixed infection cases. The larger proportion of mixed infection consisted of *E. acervulina* which may be due the wide spread distribution of this species in all management systems.

Lesion score showed significant association with clinical coccidiosis and OPG detection frequency ($p < 0.001$, $P < 0.01$ respectively). This may be explained that chicken that had lesions may more likely manifest clinical disease and also shade large amount of oocysts in droppings (Methusela, *et al.,* 2002). Thus, the lesion score in RIR breed was significantly higher than local chicken ($p < 0.05$) and that may be the mirror reflection with the frequency detection of fecal oocyst in the deep litter management system. Lesion score in local strain chicken was usually confused or overlapping with the lesions produced by gastrointestinal parasites and enteric infectious diseases. Thus, the lesion score parameter may be complicated and not reliable to indicate the level of infection and the pathogenicity of coccidial species involved with the lesion produced in the local strain chickens. *E.acervulina* and *E. maxima* occupied much greater area beyond the typical restricted sites of their species occurrence. This tendency may be explained in that the merozoites are being carried by the peristaltic and anti peristaltic waves of the intestine beyond already infected areas in heavy infection cases (Williams, (2001).

9.2. Experimental Study to Compare Natural resistance to *E. tenella* Infection

Poultry production has a paramount role in poverty alleviation and food security in the developing countries. Thus, this study might be important to search for genetically selected breed or strains, which are resistance to coccidiosis for improved poultry production at the smallholder farmer's level. Body weight difference comparison between the test and control groups was more indicative to compare the variation between the genotype chicken in the experimental infection. Hence, body weight difference in the test and control groups of the local strain chicken revealed that there was relatively higher body weight depression ($P \leq 0.01$) than the RIR breed groups. Furthermore, the test groups of local strain chicken showed significantly higher morbidity rate than RIR test groups. This result is in agreement with the work of Pinard- Van Der Laan *et al*., (1998) which in most breed lines chicken with the highest body weight depression were found to have higher mortality rate to *E. tenella* infection although this may not always true for all breed lines. Because of its quantitative nature, body weight difference as a parameter of genetic analysis is considered a better indicator in combination with other correlated parameters (Pinard_Van Der Laan, *et al*., 1998; Zhu *et a*l., 2000).

Body weight gain depression was significantly higher in the test group females of both RIR and local strain ($p \leq 0.05$, $P \leq 0.01$ respectively). However, body weight difference in test group males of RIR and local strain was not significantly different from the control groups. This observation indicates that under experimental infections males appeared to have better tolerance to body weight loss than the females. This finding was supported by Zhu *et al.,* (2000) where significant difference was obtained between sexes in relation with natural resistance to coccidiosis. However, the difference in body weight difference between male and female was not accompanied with different morbidity rate.

E. tenella infection is characterized by heavy loss of blood from the cecum and causes reduction in packed cell volume value. RBC count and haematocrit value may decrease as much as 50% on the 5^{th} and 6^{th} day of infection (Conway and McKenzie, 1991; Conway, *et al*., 1993). Thus, the change in PCV (%), in relation to natural resistance to coccidia infection, was considered in this study as a parameter to compare the natural resistance to coccidiosis. PCV is a sensitive variable to *E. tenella* infection due to excessive bleeding induced in the cecum. The reduction in PCV value (PCVr) in the test groups of both local strain and RIR breed chicken was significantly higher as compared to the control groups ($p < 0.01$, $p < 0.01$ respectively). This result showed that both genotype chickens have been subjected to high blood loss due to bleeding in the cecum. In general, comparison of PCVr value between sexes was not significant. However, the PCV reduction in the test females of local strain was significantly higher than the control group, which was different from the test male groups ($p < 0.001$, $p = 0.874$ respectively). This finding indicates the presence of variation between male and female sexes of local strain chicken in relation to susceptibility to *E. tenella*, which may be a subject for further investigation. The lowest PCV% value in this experiment was 18%, which is comparable to the minimum PCV value of 19% in 15-day-old chicks (Conway, *et al*., 1993).

Across genotype chicken total oocyst production comparison showed RIR breed shaded significantly larger number of oocysts than local strains ($p < 0.01$). Total oocyst production is related to viable epithelial cells available to the merozoites than immunity due to crowding effect in primary infection (Williams, 2001). Therefore, total oocyst output decreases in the higher oocyst inoculation dose, which is the reflection of the number of epithelial cells available for parasitisation in the intestine (Tilahun and Stockdale, 1981; Williams, 2001). On

top of higher oocyst production, RIR breed have shown significantly lower morbidity rate than the local strain chicken. This indicates that RIR breed did not show considerable clinical disease even when high number of parasites developed and high number of oocysts discharged. However, local strain chicken could not tolerate large number of oocyst inoculation and showed high morbidity but discharged low numbers of oocysts. This implies RIR breed are more resistant to *E. tenella* acute infection and this finding is in agreement with the finding of Nakai *et al*., (1993), in an experiment conducted on the susceptibility to *E. tenella* infection in partly inbred line chickens.

With regards to sex of birds, comparison in oocyst production showed that there was no significant difference between male and female. However, female test groups of RIR breed shaded significantly larger number of oocysts than males ($p < 0.05$). This result disclosed that under experimental studies males might exhibit more tolerance than females. Similar differences were also observed in body weight difference (Bwtd) in this study and it was in congruent with the finding stated by Zhu*, et al.,* (2000).

The lesion score was significantly higher in RIR than local strain chicken ($p <0.01$). However, RIR breed showed significantly low morbidity rate ($p < 0.01$) even when high lesion has produced in the intestine. This implies RIR has better tolerance to *E. tenella* infection. This finding is in agreement with the finding of Pinard-Van Der Laan, *et al.,* (1998) that high mortality rate in some breed lines showed lower lesion score in *E. tenella* acute infection. Lesion scores were positively correlated with oocyst production ($r = 0.661$). This may explain that lesion score has strong relationship with the amount of total oocyst production. High oocyst production during the peak multiplication time of the protozoa in the intestine may result in extensive epithelial cell damage in the cecum. However, lesion score values were not significantly different between male and female in both RIR and local strain chickens. The cumulative results of this experiment implies that although female test groups showed significant body weight depression and high oocyst production, there was no significant difference between male and female in resistance to *E. tenella* infection.

In relation to the specificity of natural resistance associated parameters, it has been agreed by many researchers that one or two measured parameters from chicken with coccidiosis do not truly reflect the natural resistance of an individual (Bumstead *et al*., 1991; Zhu *et al.,* 2000).

Secondly some natural resistance associated measurements are often complicated by other genetic factors that may not affect natural resistance or susceptibility. Thus, these measurements are usually weakly correlated to each other (Bumstead, *et al*., 1991; Zhu, *et al.,* 2000). This fact was also observed in this study in that oocyst production and lesion score measurements were weakly correlated with body weight difference and PCV reduction. Morbidity rate was also weakly correlated with body weight difference and PCV reduction values. However, PCV measurement was a sensitive parameter to acute *E. tenella* infection. Body weight difference observed in this study also appeared significantly important to evaluate natural resistance of chickens to coccidial infections. However, total oocyst production and lesion score measurements did not coincide with the severity of the disease through clinical manifestation although they appeared statistically positive correlated with morbidity rate ($r = 0.771$). Oocyst production and lesion score parameters coincide consistently with other measurements in challenge tests in evaluating natural resistance (Bumstead *et al.,* 1989; Medarova *et al.,* 2003). The low correlation between body weight difference and lesion score may create doubts in the usefulness of lesion scores in evaluating natural resistance (Zhu, *et al.,* 2000). From economic point of view, morbidity rate and body weight difference may be considered as a true parameter for comparison of natural resistance between the two genotype chickens in this study since these parameters determine the productivity of the chicken either to cull or retain the bird.

The Rhode Island Red breed seems to have better tolerance to cope up the effects of the disease since it showed low morbidity/ mortality and low body weight depression than local strain chicken. However, it is not sufficient to firmly conclude the resistance of this breed due to the fact that the comparison did not include more breed lines due to time limitation and difficulties to get more breed lines of chicken.

10. CONCLUSION AND RECOMMENDATIONS

The occurrence of sub-clinical coccidiosis was significantly higher than clinical coccidiosis in both genotype chicken and management systems. The birds might have developed immunity to the trickle infections acquired from the environment and maintain the state of balance to the infection in the form of sub-clinical disease. The effect of sub-clinical coccidiosis on the production performance of chicken and its economic significance should be further studied.

Coccidial infection in deep litter management was significantly higher than in the free-ranging system. This showed that management system plays a great role in the epidemiology of coccidial infection. However, the observed high prevalence rate of coccidiosis in the indigenous chicken showed that it is one of the economically important diseases, which deserve appropriate prevention and control measures.

In the current study all the pathogenic Eimeria species identified in the previous works have been identified. However, *E. mitis* was also tentatively diagnosed in this study from some samples collected from Adama poultry breeding and multiplication center and from local strain chickens too. Confirmation of this species and the distribution of this species as well as its economic importance should be studied.

Rhode Island Red breed showed less morbidity/ mortality rates and low body weight difference than local strain chicken although the birds manifested significantly higher lesion score and larger total oocyst production. This result indicated the breed might have better tolerance to cope up the effects of the disease. Moreover, the presence of higher lesion scores in this breed was not a determinant factor on the clinical manifestation of the disease and survival of the birds. This event should be further investigated.

In general terms, difference in resistance to coccidiosis, in relation to sex, was not significant. However, under experimental infections males showed better tolerance to some effects of the disease than the females. In both genotypes, female birds showed significantly higher body weight gain depression and higher oocyst production than males. PCVr value in local strain test group females was also significantly higher than the test group males, which was different

from the test group of RIR breed females. The other parameters remain similar for both sexes. This variation to the effects of coccidiosis infection should be further studied and explained. Therefore, the following specific recommendations are forwarded:

Extensive management system

- Appropriate prevention and control of coccidiosis at the smallholder farmers should be in place to alleviate the impact of the disease.
- Low-level management, housing and poor nutrition should be improved in the rural poultry production so that coccidiosis control efforts could be enhanced.
- Under the traditional management system where biosecurity measures are not practiced confinement rearing of chicken should be discouraged to reduce the risk of high coccidial oocyst accumulation and increased infection rate.
- Appropriate awareness creation, through farmers training about the general knowledge of coccidiosis occurrence, medication procedures and prevention and control methods should be undertaken for sustainable control of coccidiosis. Focus should be made on women smallholder poultry farmers in their efforts to improve income sources and long-term economic potentials.
- As far as resistance against coccidiosis is concerned Rhode Island Red breed might have better tolerance to the disease and they could be recommended for sustainable poultry development at the smallholder level.

For intensive management system:

- Biosecurity practices should be the primary concept in the prevention and control of coccidiosis. These are hygiene of poultry house personnel and premises, disinfections and litter management. Keep coccidiosis outside. Disinfect shoes before entering the chicken house. Keep wild birds and other animals out and separate older birds from younger ones.
- The birds should not be overcrowded. The feeding and watering troughs should be placed in a way that chicken can easily reach and adequate spaces are available for feeding and watering. Otherwise, contamination of feed and water with coccidia oocysts from chicken dropping is inevitable and hence increases the risk of infection and disease.

- Avoiding damp areas in house should be the practical emphasis of poultry producers. Leaking roofs or watering troughs should be adjusted; redistribute litters frequently to avoid concentration of the oocysts at places such as feeding and watering troughs. Litter should be kept dry and if possible renewed frequently.
- Clean the house thoroughly before each new flock comes in. Clean out old litter, wash the walls, floor and disinfect the room using appropriate disinfectant that can kill coccidial oocysts.
- A reduction in feed intake is a 'sure sign' of something is wrong. Thus, poultry supervisors and producers should monitor the drop in feed consumption.
- Further research is recommended on:
    - The economic importance of sub-clinical coccidiosis under both extensive and intensive management systems
    - Focused study on the possible distribution and frequency occurrence of *E. mitis* should be made
    - Natural resistance to coccidiosis should be further studied to enable breed selection and to prevent the economical losses incurred through reduced growth rate, high medicament expenses and mortalities. Moreover, substantial research work has to be done to understand the immune mechanism involved in the protection of coccidial infections and to identify gene markers responsible for coccidiosis resistance.

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12. ANNEXES

Annex 1: Mean body weight difference, PCV values, oocyst production, lesion score and morbidity rates by experimental groups, genotype chicken and sex.

No	groups	Sub groups	No of birds	Bwtd (%)	PCVa(%)	PCVr(%)	oocyst prod in (10^4)	Total morbid	Lesion score
1	LSC	Test	75	53.91[a]	33.94[a]	12.57[a]	1851[a]	0.68[a]	2.63[a]
		control	66	64.99[b]	39.26[b]	7.81[b]	0	0	0
2	RIR	Test	40	64.99[c]	34.39[c]	10.27[c]	2383[b]	0.35[b]	3.35[b]
		Control	37	79.51[d]	39.1[d]	6.45[d]	0	0	0
3	Sex	Male	101	68.33[ab]	36.81 [ac]	9.48[ab]	1926[c]	0.536[c]	2.875[c]
		Female	117	59.59[ab]	36.24 [ac]	9.83[ab]	2140[c]	0.593[c]	2.88[c]

Mean values within a column followed by different lower case superscript (a-d) are significantly different ($p<0.05$)

Annex 2: Correlations of resistance- associated parameters

		Bwtd	Pcvr	Oocypr	Totmrb	Lesscore
Bwtd	Pearson	1				
	Sig. (2-tailed)					
	N	218				
Pcvr	Pearson	-0.134*	1			
	Sig. (2-tailed)	0.32	.			
	N	218	218			
Oocypr	Pearson	-0.288**	0.115	1		
	Sig. (2-tailed)	1.17	0.09			
	N	218	218	218		
Totmrb	Pearson	-0.265**	0.416**	0.347**	1	
	Sig. (2-tailed)	0.102	1.48	1.48	.	
	N	218	218	218	218	
Lesscore	Pearson	-0.210**	0.385	0.66**	0.771**	1
	Sig. (2-tailed)		3.97	1.06	3.39	
	N	218	218	218	218	218

* Significant at the 0.05 level (2-tailed) and ** significant at the 0.01 level (2-tailed).

BWTd = Body weight gain difference

PCVr = Reduction Packed cell volume

OOCYPR = Total oocyst production

LESSCORE = Lesion score

TOTMRB = Morbidity Rate

Annex 3: Method for estimation of oocyst per gram feces (OPG) (MAFF, 1979).

1. Take 3 grams of fecal sample out of 24 hours pooled dropping and mix thoroughly with 42ml of tap water in a 120ml wide mouthed glass.

2. Pour fecal suspension through a wire mesh screen, collecting the filtrate in a clean bowl.

3. Mix the filtrate thoroughly to ensure that there is a uniform suspension of fecal material and transfer an aliquot to a centrifuge tube.

4. Centrifuge for 2 minutes at 1500 rpm.

5. Discard the supernatant fluid and emulsify the packed sediment by saturated salt solution (NaCl) until the volume equals that of the initial aliquot of filtrate.

6. Invert the tube several times until the sediment is evenly suspended and fill two chambers of a McMaster slide using a clean Pasteur pipette.

7. Count all the oocysts within the ruled area of the chambers using 10mm objective and 10x eyepiece and the average count of the two chambers were recorded. Then the mean value is calculated: (Conway and McKenzie, 1991).

$$OPG = \frac{X}{0.15} * 45 * \frac{1}{3}$$

Where X= number of oocysts counted

Vol. = 45 ml water that the feces is soaked

0.15= volume of the McMaster counting chamber

1/3 = correction factor

Annex 4: Procedure to harvest oocysts from fecal samples and preservation (Conway and McKenzie, 1991)

1. The fecal material was homogenized by blender until the fecal materials can be easily filtered through tea strainer sieve.
2. The solid materials were 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% was added into Petri dish and maintained at room temperature.

- The Petri dish was put on electric shaker and adjusted to move in a gentle motion to aerate the suspension.
- 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.

Annex 5: Procedure to estimate total oocyst production from pooled fecal sample (MAFF, 1979).

1. Feces collected in polyethylene- lined tray from each cage were made up to 1500ml with water and homogenized with an electric blender.
2. The suspensions were agitated, 5 samples of 1ml were withdrawn by a graduated pipette and add into a 75ml beaker.
3. Add saturated salt solution (NaCl) to 50ml, stir thoroughly the suspension and fill two McMaster chambers.
4. Count the oocysts in a separate two chambers and take the average count. When the concentration of the oocyst in the microscope field becomes high and difficult to count, 10x dilution of the suspension was performed and the final count was multiplied by 10. The total oocyst production (TOP) per bird in 24 hours sample was calculated by the formula:

$$TOP = \frac{X}{0.15} * vol. * 10$$

Where X= number of oocysts counted

Vol. = 1500ml water that the feces is soaked

0.15= volume of the McMaster counting chamber

10= correction factor

The number of oocysts recorded for each cage was divided by the number of chicks in that cage and expressed as the average number of oocyst produced per bird (MAFF, 1979). The average total oocyst production for each experimental chick was calculated from the sum of the three consecutive patent period oocyst discharges.

13. APPENDICES

Appendix 1: Questionnaire Format for Intensive Poultry Farms.

Please answer the following set of questionnaires by filling in the empty spaces or by marking with the cross (x) in the small box in front of the optimum answer. You may skip a question which does not apply to your farm. Your participation will be highly appreciated and all information will be treated as confidential.

A. General information

Farm locality _____

Number of farms_____ No of houses_____

Owners' name and address _____

Production system _____

Breed _____ .Production line: Broilers □; Layers □; Other (specify)_____.

Farm size: No of birds/farm_____

No of birds/ house _____

B. Information about farm history and causes of morbidity and mortality.

1. Age of the farm: 0-6 months □; 7-12 months □; 1-2 years □; more than 2 years □.
2. Purpose of keeping chicken: Family consumption □.

   Cash earning □.

   Other (specify) _____ .
3. Disease problem in the farm: yes/no. If yes:
   I. Major diseases in your farm (in order of importance):

      A)_____ B)_____

      C)_____ D)_____

   II. Number of birds which get sick due to each of the above diseases:

      A)_____ B)_____

C) _____ D)_____

4. Deaths of birds in your farms. Yes/ No. If yes:

I. Major causes of deaths (in order of importance):

A)_____ B)_____

C) _____ D)_____

II. Number of birds which die due to each of the above causes:

A)_____ B)_____

C)_____ D)_____

C) **Information about disease control measures.**

5. Disease control measures in your farm:

Drugs □; Cleaning and disinfection □; Vaccination □; Other (specify)_____.

6. How long have you been using the above measures?

1 year □; 2 years □; more than 2 years □.

7. Frequency of manure disposal: Once/ week □; Once/ month □; Once/ batch □;

Other (specify)_____ .

8. Drugs used in the farm starting with the most frequently used.

A) _____ B)_____

C)_____ D)_____

9. Level of biosecurity measures in the farm:

i. Presence of car wheel bath disinfectant at the gate Yes/ No.

ii. Presence of foot bath disinfectant at each poultry house Yes/ No.

iii. Its regular functioning. Good □; satisfactory □;Poor □.

iv. The hygiene control level of poultry house attendants and supervisors to keep the sanitation. Good □; satisfactory □; poor □.

v. Any cracks, holes or opening to the poultry houses through which wild birds, rodents or predators enter into the houses. Maximum □; Minimum □; No □.

D. Information about the Production.

10. Source of Day-Old-Chicks: Own □; Local supplier □; Imported □.

11. Age and weight at which birds are sold: Age □; Weight □.

12. Culling birds from the farm before sale age Yes/ No. If yes:

Number culled_____.

Reason for culling_____.

13. Age at which birds start laying: _____.

E. Information about feed and feeding.

14. Source of feed:

□ manufactured in the farm.

□ brought from local suppliers.

Other (specify)_____.

15. Feed management

□ Ad libitum

□ Controlled

Other (specify)_____.

16. Quality and availability of feed: Good □; Satisfactory □; Poor □.

17. Period of illumination per day (proportion of light/ dark out of 24 hours):

Broilers- light/ dark _____/_____Hrs

Layers- Light/ dark_____/_____Hrs.

18. Quality of feeding and watering equipment in relation to feeding and watering spillage:

Good □; Satisfactory □; Poor □.

19. Type of feeding system: Automated □; Manual □.

20. Use of coccidiostats: Yes □; No □; Sometime □.

21. Method of application: In feed □; In water □; In feed and water □.

F) Information about Environment/ Management Factors.

22. Litter moisture: Average □; High □.

23. Stocking density (Number of birds per meter square).

In deep litter-Broilers _____

Layers_____

In Cage system- Layers _____

24. Ventilation system: Good □; satisfactory □; Poor □.

G) Information about Coccidiosis.

25. Coccidiosis in the farm. Yes□; No□. If yes:

When the problem did starts? _____

Effect on the farm performance. High □; Medium □; Low □.

Number of birds currently showing signs _____

Number of birds which die due to Coccidiosis per batch out of initial No of DOC____.

Measures used to control the disease:

A)_____ C)_____

B)_____ D)_____.

26. Have your control measures been continuous? Yes □ No □

Appendix 2. Questionnaire Format for Smallholder Poultry Farmers in the Study Area

The under seen questions are designed to be replied by the farmers who undertake poultry production either traditionally or improved system. Please answer the following set of questionnaire by filling in the empty space with the cross (x) mark in front of the best answer or write a short answers in the space provided. Your participation will be highly appreciated.

1. Study locality:
    a. Region - Oromia
    b. Zone - Arsi
    c. Wereda- Tiyo
    d. Kebele (Peasant Association)_____
2. Interviewed farmers Name _____ sex_____Age____
   Marital status: Married____ Unmarried____ Others__
3. Altitude of the PA's _____
4. Agro-climatic condition of the PA: Frost highland_____ High land_____ Midland_____ Lowland_____ .
5. Farming system: Crop production_____ Livestock production_____ Mixed farming_____ .
6. Types of crops produced in the PA. List them in order of importance from high to lower rank. 1st_____2nd_____3rd_____4th_____5th_____.
7. Livestock population in order of most dominant number to lower number. 1st_____2nd_____3rd_____4th_____5th_____
8. Number of chicken in your house: <2_____, 3to 5_____, 6 to 10_____, 11 to 20_____, >21_____.
9. For how long have you worked in poultry production? < 6 months_____, 6 months to 1 year_____, 1 -2 years_____, >2 years_____.
10. Among the family members, poultry production is a duty of: House wife_____, House holder_____, Children_____.
11. Income generated from poultry production is liable to be used by: House holder_____, House wife_____, Children_____.
12. What type of poultry production system do you follow? Traditional_____, Improved_____, Modern_____.
13. The purpose of keeping birds: For house consumption_____, For market_____, For both_____.

14. Feeding of chicken:
    a. The chicken feed by scavenging only around the house
    b. Supplemented with cereal grain and food residuals in addition to scavenging.
    c. Commercially available feed in a certain confined place
    d. Others (mention)_____

15. Over night accommodation: Properly built house_____, small hat_____, simple wooden bar_____.
16. Nesting place: Well prepared place_____, Low quality prepared place_____, No prepared place_____.
17. Poultry disease occurrence in the area: Very high_____, High_____, Medium_____, No disease_____.
18. The clinical signs manifested by chicken sick/ died with disease in order of descending (in relative terms) : (Diarrhea, coughing & nasal discharge, torticolis, ectoparasites, wound in the head region, swelling in the head region).
    1st_____,2nd_____,3rd_____,4th_____,5th_____.
19. Seasonal disease occurrence observation in chicken: Yes_____, No, (occurs always)_____.
20. If the answer is 'yes' which clinical sign among the above (no, 18) follow seasonal occurrence (in order of descending): 1_____, 2_____, 3_____, 4_____,5_____.
21. In which season do you observe high disease occurrence:
    In summer_____, In winter_____, In short rainy season_____
22. Which age groups are more susceptible to disease?
    Chicks_____,Pullets_____, Old age groups_____.
23. What measures do you take during poultry disease outbreak?
    a. Visit vet. clinic and treat as rep the recommendation drug
    b. Apply traditional medicine
    c. Sale infected birds
    d. Others (mention)_____.

24. What types of drugs did you get from vet. Clinic? (mention the most important ones you remember): 1_____2_____3_____4_____

25. List down the names of traditional medicines you know or utilize for poultry disease in order of descending:
    a.
    b.
    c.
    d
    e.
26. Which type of diarrhea do you observe frequently in chicken (in order of descending): Bloody diarrhea_____,Chalky diarrhea_____, Greenish diarrhea_____.
27. In which season does bloody diarrhea frequently be observed? In summer_____, In winter_____, Always_____.
28. Which season do you prefer to brood hens and manage chicks? In summer_____, In winter_____, Always_____.
29. From your observation, which type of chicken (local strain chicken or Rhode Island Red breed) is more resistance to disease occurrence? Local strain_____ Rhode Island Red breed_____.
30. Which disease type (s) more frequently affects Rhode Island Red breed? List down in order of descending from the clinical signs mentioned under no. 18.
    1^{st}_____2^{nd}_____3^{rd}_____4^{th}_____5^{thh}_____.

Appendix 3: ANOVA Model for Analysis of Experimental Study

1. anova bwtd breed sex group breed* sex breed* group sex* group

```
                  Number of obs =     218     R-squared     =  0.1375
                  Root MSE      = 26.0805     Adj R-squared =  0.1130

    Source |     Partial SS             df        MS               F        Prob > F
-----------+----------------------------------------------------------
     Model |      22876.2916            6     3812.71527         5.61     0.0000
           |
     breed |       7462.10881           1     7462.10881        10.97     0.0011
       sex |      3123.96068            1     3123.96068         4.59      0.0333
     group |       8081.93699           1     8081.93699        11.88     0.0007
  breed*sex |        1.68510729          1     1.68510729         0.00     0.9604
breed*group |        16.201072           1      216.201072        0.32     0.5735
  sex*group |      2493.58495            1     2493.58495         3.67     0.0569
           |
   Residual |  143521.038    211  680.194491
-----------+----------------------------------------------------------
      Total |  166397.329    217  766.807969
```

.

2. anova pcva breed sex group breed* sex breed* group sex* group

```
                  Number of obs =     218     R-squared     =  0.4031
                  Root MSE      = 3.25402     Adj R-squared =  0.3862

    Source |     Partial SS    df       MS          F          Prob > F
-----------+----------------------------------------------------------
     Model |     1509.06554     6  251.510923      23.75     0.0000
           |
     breed |      .521546413    1  .521546413      0.05     0.8246
       sex |      27.9372338    1  27.9372338      2.64     0.1058
     group |      1233.73711    1  1233.73711     116.52     0.0000
  breed*sex |     .054870726    1  .054870726      0.01     0.9427
breed*group |     2.87367663    1  2.87367663      0.27     0.6029
  sex*group |     51.4959194    1  51.4959194      4.86     0.0285
           |
   Residual |  2234.20273    211  10.5886385
-----------+----------------------------------------------------------
      Total |  3743.26826    217  17.2500842
```

3. anova oocypr breed sex group breed* sex breed* group sex* group

```
      Number of obs =     218     R-squared     =  0.7073
      Root MSE      = 678.281     Adj R-squared =  0.6990

      Source |           Partial SS    df       MS            F       Prob > F
-------------+----------------------------------------------------------------
       Model |          234548530     6    39091421.7    84.97     0.0000
        breed |         3616325.04     1  3616325.04      7.86     0.0055
         sex |          1022958.99     1  1022958.99      2.22     0.1374
       group |           220987845     1    220987845    480.34     0.0000
  breed*sex |          327795.414     1   327795.414      0.71     0.3996
breed*group |          3748975.08     1   3748975.08      8.15     0.0047
  sex*group |          807485.735     1   807485.735      1.76     0.1867
            |
    Residual |         97073692.7   211  460064.894
-------------+----------------------------------------------------------------
       Total |          331622223   217  1528213.01
```

4. anova oocypr sex group sex* group if breed==1

```
      Number of obs =     141     R-squared     = 0.6060
      Root MSE      = 756.342     Adj R-squared = 0.5974

      Source |          Partial SS    df       MS            F     Prob > F
-------------+----------------------------------------------------------------
       Model |          120556662     3    40185554      70.25     0.0000
         sex |          155325.224     1  155325.224       0.27     0.6032
       group |           116471785     1   116471785     203.60     0.0000
   sex*group |          155325.224     1  155325.224       0.27     0.6032
    Residual |          78371288.8   137 572053.203
-------------+----------------------------------------------------------------
       Total |          198927951   140 1420913.93
```

5. anova oocypr sex group sex* group if breed==2

```
      Number of obs =      77     R-squared     = 0.8578
      Root MSE      = 502.169     Adj R-squared = 0.8520

      Source |           Partial SS    df       MS            F     Prob > F
-------------+----------------------------------------------------------------
       Model |          111086548     3 37028849.3     146.84     0.0000

         sex |          945912.645     1  945912.645       3.75     0.0566
       group |           109964783     1   109964783     436.07     0.0000
   sex*group |          945912.645     1  945912.645       3.75     0.0566
    Residual |          18408651.7    73  252173.311
-------------+----------------------------------------------------------------
       Total |          129495200    76  1703884.21
```

6. anova oocypr breed sex group breed* sex breed* group sex* group

```
      Number of obs =     218     R-squared     =  0.6705
      Root MSE      = 915.163     Adj R-squared =  0.6612

  Source |       Partial SS    df       MS           F         Prob > F
-----------+------------------------------------------------------------
   Model |      359646634     6  59941105.7      71.57     0.0000
         |
   breed |      20878167.2     1  20878167.2      24.93     0.0000
     sex |      4156441.5     1  14156441.5      16.90     0.0001
   group |      303753921     1   303753921     362.68     0.0000
breed*sex |      12223173.4     1  12223173.4      14.59     0.0002
breed*group |      22870816.3     1  22870816.3      27.31     0.0000
sex*group |      8835478.67     1  8835478.67      10.55     0.0014
         |
Residual |      176717251   211  837522.515
-----------+------------------------------------------------------------
   Total |      536363885   217  2471722.97
```

7. anova lesscore breed sex group breed* sex breed* group sex* group

```
      Number of obs =     218     R-squared     =  0.7884
      Root MSE      = .768216     Adj R-squared =  0.7824

  Source |       Partial SS    df       MS           F         Prob > F
-----------+------------------------------------------------------------
   Model |      463.903597     6   77.3172662     131.01     0.0000
         |
   breed |      6.45154404     1  6.45154404      10.93     0.0011
     sex |      .052674787     1  .052674787       0.09     0.7654
   group |      441.330947     1  441.330947     747.82     0.0000
breed*sex |      .066156956     1  .066156956       0.11     0.7381
breed*group |      6.52819222     1  6.52819222      11.06     0.0010
sex*group |      .028697777     1  .028697777       0.05     0.8257
         |
Residual |      124.523008   211  .590156436
-----------+------------------------------------------------------------
   Total |      588.426606   217  2.71164334
```

8. anova totmrb breed sex group breed* sex breed* group sex* group

```
                   Number of obs =     218     R-squared     =  0.4120
                   Root MSE      = .351611     Adj R-squared =  0.3953

      Source |       Partial SS    df       MS           F     Prob > F
-------------+----------------------------------------------------------
       Model |    18.2809736     6   3.04682894     24.64     0.0000
             |
       breed |     .98643394     1    .98643394      7.98     0.0052
         sex |     .082862352    1   .082862352      0.67     0.4139
       group |     12.1209806    1   12.1209806     98.04     0.0000
   breed*sex |     .029962757    1   .029962757      0.24     0.6230
 breed*group |     1.00705939    1   1.00705939      8.15     0.0047
   sex*group |     .120303067    1   .120303067      0.97     0.3250
             |
    Residual |  26.0859988   211   .123630326
-------------+----------------------------------------------------------
       Total |  44.3669725   217   .204456094
```

9. logistic totmrb breed sex group breed* sex breed* group sex* group

```
note: group~=1 predicts failure perfectly
      group dropped and 103 obs not used

note: breed dropped due to collinearity
note: sex dropped due to collinearity
note: breed dropped due to collinearity
note: group dropped due to collinearity
note: sex dropped due to collinearity
note: group dropped due to collinearity

Logit estimates                           Number of obs   =        115
                                          LR chi2(2)      =      11.84
                                          Prob > chi2     =     0.0027
Log likelihood = -72.808714               Pseudo R2       =     0.0752

------------------------------------------------------------------------------
      totmrb | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       breed |   .2554001   .1058015    -3.29   0.001     .1133976    .5752252
         sex |    1.198975   .4762689     0.46   0.648      .550411    2.611758
------------------------------------------------------------------------------
```

10. logistic totmrb breed sex breed* sex if group==1

note: breed dropped due to collinearity
note: sex dropped due to collinearity

Logit estimates Number of obs = 115
LR chi2(2) = 10.06
Prob > chi2 = 0.0065
Log likelihood = -74.328791 Pseudo R2 = 0.0634

totmrb	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
breed	.3064973	.1261677	-2.87	0.004	.1367839	.6867811
sex	1.52036	.5950404	1.07	0.284	.7059959	3.274091

11. anova totmrb breed sex breed* sex if group==1

Number of obs = 115 R-squared = 0.0880
Root MSE = .484528 Adj R-squared = 0.0634

Source	Partial SS	df	MS	F	Prob > F
Model	2.51476517	3	.838255058	3.57	0.0164
breed	2.10976688	1	2.10976688	8.99	0.0034
sex	.174125334	1	.174125334	0.74	0.3910
breed*sex	.056813725	1	.056813725	0.24	0.6237
Residual	26.0591479	111	.234767098		
Total	28.573913	114	.25064836		

12. anova oocypr sex group group* sex if breed==2

Number of obs = 77 R-squared = 0.7242
Root MSE = 1094.15 Adj R-squared = 0.7128

Source	Partial SS	df	MS	F	Prob > F
Model	229449198	3	76483066.1	63.89	0.0000
sex	19633886.0	1	19633886.0	16.40	0.0001
group	194458651	1	194458651	162.43	0.0000
group*sex	19633886.0	1	19633886.0	16.40	0.0001
Residual	87392229.3	73	1197153.83		
Total	316841428	76	4168966.15		

13. anova lesscore sex group group* sex if breed==2

```
      Number of obs =      77     R-squared     =  0.9354
      Root MSE      = .452047     Adj R-squared =  0.9327

  Source |       Partial SS    df       MS          F     Prob > F
-----------+------------------------------------------------------
   Model |       215.887902     3   71.9626339     352.16     0.0000
         |
     sex |       .087874998     1   .087874998       0.43     0.5140
   group |       215.799298     1   215.799298    1056.05     0.0000
group*sex |       .087874998     1   .087874998       0.43     0.5140
         |
Residual |  14.9172932    73  .204346483
-----------+------------------------------------------------------
   Total |  230.805195    76  3.03691046
```

14. anova totmrb sex group group* sex if breed==2

```
      Number of obs =      77     R-squared     =  0.5691
      Root MSE      = .051554     Adj R-squared =  0.5514

  Source |       Partial SS    df       MS          F     Prob > F
-----------+------------------------------------------------------
   Model |       .256217334     3   .085405778      32.13     0.0000
         |
     sex |       .000866425     1   .000866425       0.33     0.5698
   group |        .25549921     1    .25549921      96.13     0.0000
group*sex |       .000866425     1   .000866425       0.33     0.5698
         |
Residual |  .194023789    73   .00265786
-----------+------------------------------------------------------
   Total |  .450241123    76  .005924225
```

15. anova oocypr sex group group* sex if breed==1

```
      Number of obs =     141     R-squared     =  0.6060
      Root MSE      = 756.342     Adj R-squared =  0.5974

  Source |       Partial SS    df       MS          F     Prob > F
-----------+------------------------------------------------------
   Model |       120556662     3   40185553.9      70.25     0.0000
         |
     sex |       155325.224     1   155325.224       0.27     0.6032
   group |       116471785     1    116471785     203.60     0.0000
group*sex |       155325.224     1   155325.224       0.27     0.6032
         |
Residual |  78371288.8   137  572053.203
-----------+------------------------------------------------------
   Total |   198927951   140  1420913.93
```

16. anova lesscore sex group group* sex if breed==1

```
      Number of obs =     141     R-squared     =  0.6886
      Root MSE      = .894209     Adj R-squared =  0.6818

  Source |       Partial SS    df       MS          F     Prob > F
-----------+------------------------------------------------------
   Model |       242.212437     3    80.7374789     100.97     0.0000
         |
     sex |        .000108987     1   .000108987       0.00     0.9907
   group |       235.831095     1    235.831095     294.93     0.0000
group*sex |       .000108987     1   .000108987       0.00     0.9907
         |
 Residual |  109.546429   137  .799608968
-----------+------------------------------------------------------
   Total |  351.758865   140  2.51256332
```

17. anova oocypr sex group group* sex if group==1& breed==2

```
      Number of obs =      40     R-squared     =  0.3184
      Root MSE      = 1516.51     Adj R-squared =  0.3005

  Source |       Partial SS    df       MS          F       Prob > F
-----------+------------------------------------------------------
   Model |      40822121.3     1  40822121.3      17.75     0.0001
         |
     sex |      40822121.3     1  40822121.3      17.75     0.0001
   group |      0.00     0
group*sex |       0.00     0
         |
 Residual |  87392229.3    38  2299795.51
-----------+------------------------------------------------------
   Total |   128214351    39  3287547.45
```

18. anova oocypr sex group group* sex if group==1& breed==2

```
      Number of obs =      40     R-squared     =  0.3184
      Root MSE      = 1516.51     Adj R-squared =  0.3005

  Source |       Partial SS    df       MS          F     Prob > F
-----------+------------------------------------------------------
   Model |      40822121.3     1   40822121.3      17.75     0.0001
         |
     sex |      40822121.3     1  40822121.3      17.75     0.0001
   group |      0.00     0
         |
 Residual |  87392229.3    38  2299795.51
-----------+------------------------------------------------------
   Total |   128214351    39  3287547.45
```

Appendix 4: CURRICULUM VITAE

1. **General**

1.1. Name Getachew Gari

1.2. Date of Birth : Augest,1965

1.3. Place of Birth : Obera, Bale Zone, Oromia Regional State

1.4. Marital Status : Married

1.5. Nationality : Ethiopian

1.6. Language ability : English Writing and speaking
Amharic writing and speaking
Oromiffa writing and speaking

1.7. Current Address : P.o.box 212 Arsi- Asella
Tel. 02-312858 Res.
02-311324 Off.

2. Educational Background

2.1. Grade 1-8 Oberra Junior School, Bale

2.2. Grade 9-12 Batu Terara Senior Secondary School, Bale – Goba

2.3. Higher Education- AAU- FVM Debre Zeit

3. Qualification

3.1. Doctor of Veterinary Medicine (DVM) from AAU- FVM in 1989 GC

4. Work Experience

4.1. Team Leader of Wereda Agricultural Office Animal and Fisheries Resources Development 1990-1996

4.2. Team Leader of Bale Zone Agricultural Department Animal and Fisheries Resources Development 1996-1997

4.3. Deputy Head Regulatory Division of Bale Zone Agricultural Department 1997-2001

4.4. Asella Regional Vet. Laboratory, Histopathology Team Leader 2001-2003

5. Additional Skill

5.1. Diploma in Computer Service

5.2. Second grade Driving License

6. Trainings and Workshops

6.1. Render pest Surveillance and Sero- surveillance training

6.2. Human Resource Management Training

7. Research Work

Camel GIT Helminthiasis- DVM Thesis

8. References

1. Dr. Hailu Wondimu, Head of Asella Regional Vet. Laboratory

   Address- P.o. Box 212, Tel- 02-311324 Office

2. Dr. Getachew Tilahun, Pathobiology Institute

   Address- Tel- 01-135728