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HEMATOLOGICAL AND PATHOLOGICAL STUDY ON CHICKENS NATURALLY INFECTED BY COCCIDIOSIS IN AND AROUND AMBO TOWN, WEST SHEWA ZONE, OROMIA REGIONAL STATE, ETHIOPIA

MSc Thesis

By

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June, 2015

Bishoftu, Ethiopia

HEMATOLOGICAL AND PATHOLOGICAL STUDY ON CHICKENS NATURALLY INFECTED BY COCCIDIOSIS IN AND AROUND AMBO TOWN, WEST SHEWA ZONE, OROMIA REGIONAL STATE, ETHIOPIA

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

By

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by Morka Dandecha entitled **"Hematological and Pathological studies on chickens naturally infected by coccidiosis in and around Ambo town, west Shewa zone, Oromia regional state, Ethiopia"** and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Tropical veterinary pathology.

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DEDICATION

I dedicate this manuscript to my beloved brother “Ato Bonsa Dandecha”, who passed away accidentally without seeing any of my achievements.

STATEMENT OF AUTHOR

I declare that the thesis hereby submitted for the MSc degree at the Addis Ababa University, College of Veterinary Medicine and Agriculture is my own work and has not been previously submitted to any other University or institution for the award of any degree. I concede copyright of the thesis in favor of the Addis Ababa University, Collage of Veterinary Medicine and Agriculture.

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LIST OF ABBREVIATIONS

AARDB	Ambo agricultural and rural development bureau
CI	Confidence Interval
CSA	Central statistics authority
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and agriculture organization
GLS	Gross lesion scoring
GIT	Gastrointestinal tract
Hb	Hemoglobin Concentration
IB	Isa brown
LB	Local breed
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MLS	Microscopic lesion scoring
NAHDIC	National animal health diagnostic and investigation center
PCR	Polymerase chain reaction
PCV	Packed Cell Volume
PKB	Potchefstroom Koekoek Breed
RBC	Red blood cells
RNA	Ribonucleic acid
SD	Standard deviation
SPSS	Software program for Social Science
WBC	White Blood Cells

ABSTRACTS

A cross sectional study was conducted from November 2014 to April 2015 with the objective to determine hematological and pathological changes on local, Isa brown and koekoek breeds of chickens naturally infected with coccidosis. The study involved hematological, post mortem, mucosal scraping, gross and histopathological examinations. A total of 113 chickens with clinical signs suggestive of coccidiosis were examined. Five *Eimeria* species namely: *E. tenella* (48.8%), *E. necatrix*, (11.9%), *E. brunetti* (10.4%), *E. maxima* (6%) and *E. acervulina* (3%) were identified. Reduction on the red blood cells, Hemoglobin and decreased packed cell volume values were seen due to the effect of the parasites. From a total of 113 chickens examined, 67 (59.3%) were positive for *Eimeria* species. Fifty three (79.1%) of the coccidian positive chickens, showed visible gross lesions in the intestine and caecum. The frequency of detection of gross lesions in koekoek breed was significantly higher than that of local and Isa brown breeds (χ^2 = 20.731, $p < 0.05$). Comparisons were made between microscopic & gross lesions from small intestine and caecum and it was found that certain lesions that were graded as mild in gross examinations showed significant microscopic lesion. Histopathological examinations of the affected small intestine and caecum showed excessive tissue damage, severe hemorrhagic enteritis with epithelial necrosis, presence of large clusters of schizonts and meronts in the damaged epithelial cells along with infiltrating inflammatory cells especially of eosinophils. In conclusion, the present study showed that coccidiosis had a destructive effect on chickens that is represented by a high reduction in red blood cell, packed cell volume, hemoglobin, increment in leukocyte counts and various gross and microscopic lesions. Prevention and control methods need to be implemented to reduce the loss due to coccidiosis.

***Key words**: Ambo, Chickens, Coccidosis, Eimeria, Ethiopia, Hematology, Lesion*

1. INTRODUCTION

Poultry production has been constantly increasing over the past decades and a survey made by FAO shows that the world poultry population has been estimated to be about 16.2 billion, with 75% in developing countries, producing 67, 718,544 metric tons of chicken meat and 57,861,747 metric tons of hen eggs per annually (Gueye, 2005). Over the years there has been an increasing demand for poultry products both for nutritional supply and poverty alleviation in the village communities. Chicken and eggs provide an important source of animal protein for poor families and give significant cash income when sold at the market (Kuit *et. al.*, 1986; Pandey *et al.*, 1992).

In Africa, village poultry contributes over 70% of poultry products and 20% of animal protein intake. In East Africa, over 80% of human population live in rural areas and over 75% of these households keep indigenous chickens and Ethiopia is not exceptional from this situation (Kitalyi, 1998). The poultry population in Ethiopia has been estimated to be 34.2 million. Out of the total population, 99% consisted native chickens and managed in a scavenging system while the remaining birds are mainly kept in private farms under a modern management system. In Ethiopia, poultry farming is being progressed form a traditional backyard rearing to an organized small-scale and medium scale commercial venture in the last decade. Currently, there is huge demand of poultry meat and eggs which is higher as compared to the supply (FAO, 1998; Saferi *et a*l., 2004). To enable to feed her people and ensure food security as one option, Ethiopia needs to improve the livestock sector through intensification particularly the poultry production sector. The poultry sector has the potential to provide relatively low priced or economical animal protein to the population and improve the nutritional status, to create both rural and urban employment and to generate income in time of economic difficulty (Tadelle *et al.,* 2003; CSA, 2011).

However, the poultry farming has been adversely affected by a variety of constraints (FAO, 1998). Of these constraints, poultry diseases and health problems continue to play the major central role in hampering its development, value and profitability, particularly

to small-scale poultry farming. Among the infectious diseases of poultry, coccidiosis is the major parasitic disease (FAO, 1998; Rushton *et al*., 1999).

Coccidiosis in chickens is a major problem of poultry industry that is caused by the intracellular protozoan parasite of genus the *Eimeria* (Taylor *et al*., 2007). The disease is endemic in most of the tropical and subtropical regions where ecological and management conditions favor an all-year round development and propagation of the causal agent (Obasi *et al*., 2006). Velker (2011) reported that nine species of *Eimeria* have been described in chickens, of which at least seven species are relevant for the poultry industry (*E. acervulina, E. maxima, E. brunetti, E. necatrix, E. mitis, E. praecox and E. tenella*).

The occurrence of clinical coccidiosis is directly related to the number of sporulated oocysts ingested by poultry at one time, the pathogenicity of the *Eimeria* species, the age of the infected chicken and the management system. Coccidiosis is primarily causing severe enteritis with hemorrhages, tissue damage, anemia and some metabolic disturbances (Panda *et al*., 1997; Deger *et al*., 2002; Onyach-Olaa, 2003). Symptoms of the disease start to appear at the time when the second generations of schizonts start to replicate, grow, mature and release the second generation of merozoits. Second generation of merozoits cause inflammation of the sub epithelial mucosa, desquamation of the epithelia and capillary rupture in the intestinal wall. As a consequence, bloody diarrhea occurs (Jordan, 1990; Abouzeid *et. al*., 2010).

In Ethiopia, available evidences witness that poultry mortalities due to diseases are estimated to be 20-50% where poultry coccidiosis is one of the major diseases causing significant poultry losses(Lobago *et al* ., 2005). It is used to be the most important cause of mortalities in all farms and yet is a problem as reported by various investigators (Fessesse-work, 1990; Kalifa, 1997; Ashenafi, 2000; Methusela, 2001; Safari *et al*., 2004).

Farmers in and around Ambo town have embraced chicken rearing activity, but a number of farmers were complaining of chicken mortalities especially at early age, stunted growth and poor weight gain. Prevalence of poultry coccidiosis was assessed in local and Rhode Island breeds in the study area (Shiferaw, 2014). However, researches have not been done on prevalence, hematological and pathological changes of Isa brown and Koekoek breeds infested by *Eimeria* species in Ethiopia. Only little information were available in the literature concerning the evaluation of hematological alterations in chickens infected with E*imeria* species. The few studies which have analyzed hematological parameters are restricted to the leukocyte profile and type of immune response to infection caused by the parasite (Irizaary-Rovira, 2004).

Therefore, the objectives of this study were:

General objective

- ✓ To assess chicken coccidiosis and to provide information on hematological and pathological changes of chickens infected with coccidiosis in the study area.

Specific objectives

- ❖ To identify Eimeria species circulating in the study area
- ❖ To characterize gross and histopathological lesion in chickens infested with coccidiosis.
- ❖ To explore changes in hematological values (total RBC and WBC count, PCV, Hb) and red blood cell indices (MCV, MCH and MCHC) of chickens positive to coccidioisis.

2. LITERATURE REVIEW

2.1. Etiology

Causative agent of the disease belong to phylum Apicomplexa, class Sporozoa, subclass Coccidia, order Eucoccidia, suborder Eimerinae, and family Eimeridae, genus *Eimeria.* Seven species of *Eimeria* (*E. acervulina, E.brunetti, E. maxima, E. mitis, E. necatrix, E. praecox* and *E. tenella*) are recognized as infecting chickens. Depending on the localization, disease in poultry has two forms, coccidiosis of the caecum that is caused by *Eimeria tenella* and intestinal coccidiosis that is caused by *E. necatrix, E. acervulina, E. maxima, E. brunetti, E. mitis and E. praecox* (Chauhan**,** 2010**)**. *E. tenella* and *E. necatrix* are the most pathogenic species (Soulsby, 1982).

Eimeria species are frequently described by the morphology of the oocyst. Oocysts are enclosed in a thick outer shell and consist of a single cell that begins the process of sporulation to yield the infective stage in about 48 hours (Saif *et al*., 2003**)**.

2.2. Life cycle

Development of the parasite in the host cells involves both asexual and sexual stages of multiplication. A generalized life cycle is illustrated in Figure 1. Infection occurs when a susceptible chicken ingests a sporulated oocyst from its environment. The sporulated oocyst contains four sporocysts, each sporocysts contains two sporozoites. The sporozoites are released by mechanical and biochemical action in the digestive tract of the chicken **(**Saif *et al*., 2003**)**. The liberated sporozoites invade epithelial cells in a specific zone of the intestine or caecum depending on the species. Upon entering the host cell, the sporozoites transforms in 12 to 48 hours to a feeding stage called a trophozoite. The trophozoite begins to enlarge, and the parasite nucleus divides by a process of asexual multiple divisions known as schizogony (merogony). At this point, the parasite stage is referred to as a schizont or meront. The schizont ruptures when mature in third day, releasing the merozoits. Most of these invade other epithelial cells to repeat the

process of development through the trophozoite and schizogonous stages. The merozoits from the second schizogonous cycle again penetrate the epithelial cell of the host. Some or all may go through a third schizogonous cycle, depending on the species, before formation of micro gametocytes (male) or macro gametocytes (female). The male gametocyte matures and ruptures, releasing a large number of minute biflagellate microgamete. The macro gametocyte grows to form a macrogamete (Zander and Mallinson, 1991).

A thickened wall forms around the macrogamete, forming a zygote when the macrogamete is fertilized by a microgamete. This stage is the young or immature oocyst. The prepatent period varies with each species depending on the time required for each schizogonous cycle and the number of cycles. The oocyst ruptures the host cell when mature and passes out of the bird in the droppings (Zander and Mallinson, 1991).

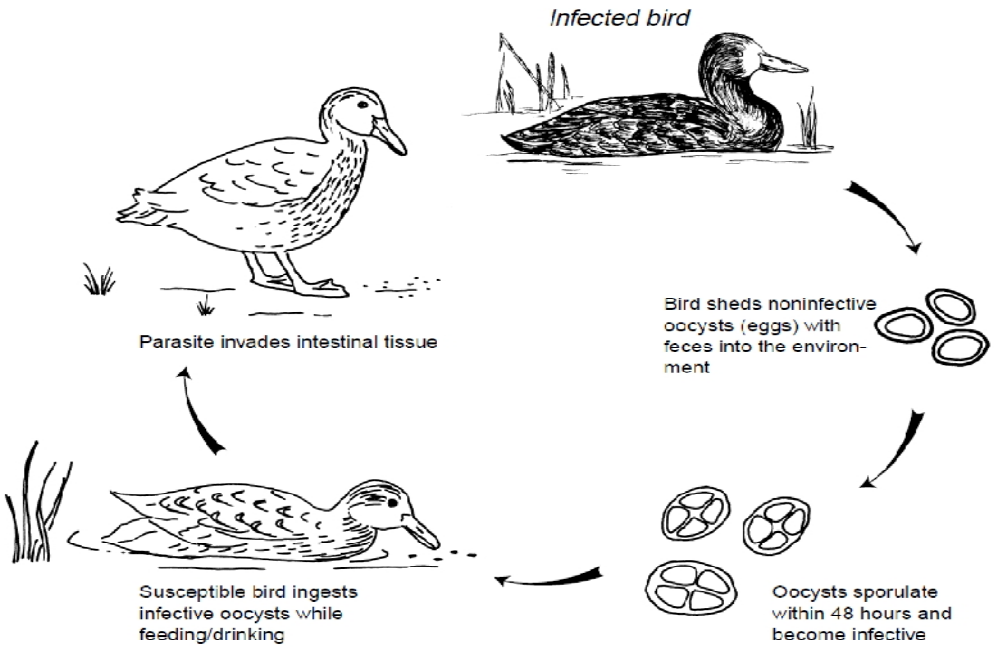

Figure 1 : Direct life cycle of Eimeria infection in birds (Conway and McKenzie, 2007)

2.3. Epidemiology

2.3.1. Distribution

Coccidiosis occurs worldwide and is a major cause of mortality and suboptimal growth and feed conversion efficiency in immature flocks unless appropriate preventive measures are implemented. Coccidia have been found wherever poultry are raised. The spread of the parasite is enhanced by poor bio-safety and management practices **(**Simon, 2005**)**.

Distribution and prevalence is influenced by several factors: high animal density cramped on a small space, high air temperature, high relative humidity, different (especially different age) categories of birds at same place, feed change, quality of feed, as well as all other factors that compromise resistance to the disease and general health status of the birds (Calnek, 1997). Onset of the disease depends on the age of the bird at the time of first infection and number of passages of the infective stage of oocyst (for one passage to be completed it is required 10 days), as well as on ability of the bird to develop proper specific immune response (Ilic *et al.*, 2003; Williams, 2005).

The highest incidence of coccidiosis is during spring and fall, especially when weather is cold and humid (rain). The incidence is significantly smaller during hot and dry weather conditions. The intensity of the infection depends on the number of Oocysts that are ingested and the immune status of the bird (Calnek, 1997). Infection of young chicken cannot be avoided in intensive production systems, whatever prophylactic measures have been taken. So, infection takes place in the first weeks of life. Intensive poultry production systems, high density of totally susceptible birds and many passages of the causative agent in the new bird generation, pose almost ideal circumstances for infection to persist and spread within the flock (Razmi and Kalideri; 2000). Heavy load of infectious Oocysts on the floor is one of the most important prerequisite conditions for infection to persist in the flock (Jordan, 1990).

In Ethiopia coccidiosis is the most important cause of mortalities in all farms. Incidences of the disease were as higher as 80%**,** usually occurring in the form of outbreaks**.** Poultry coccidiosis caused by *E. acervulina*, *E. necatrix*, *E. maxima* and *E. tenella* is endemic in all parts of the country and affects mainly young birds. It is a major cause of both direct and indirect losses in all farms due to mortalities, coccidiostat costs, reduced weight gains, reduced market value of affected birds, delayed off take and reduced egg production in layers (Safari *et al.*, 2004**)**.

2.3.2. Transmission

Chickens become infected with *Eimeria* species by ingesting infective oocysts (eggs) from litter, soil and contaminated feed and water. The infected birds excrete oocysts into their feces and are a source of infection for other birds. Infected chickens may shed Oocysts in the feces for several days or weeks. The Oocysts in feces become infective through the process of sporulation in about two days **(**Khan *et al*., 2006**)**. Ingestion of viable sporulated Oocysts is the only natural method of transmission. Oocysts can be spread mechanically by many different animals, insects, contaminated equipment, wild birds, and dust **(**Jeurissen *et al*., 1996; Ilic *et al.*, 2003; Simon, 2005).

Oocysts may survive for many weeks under optimal conditions but will be quickly killed by exposure to extreme temperatures or drying. Exposure to 55°C or freezing kills Oocysts very quickly. Even 37°C kills Oocysts when continued for 2-3 days (Sherif *et al*., 2008).

2.4. Pathogenesis

The infectious forms of the causative agent are Oocysts in the form of spores. Infection is by oral route, with contaminated feed and/or water. After ingestion, infectious Oocysts excist, liberating the infective form sporozoites. Sporozoite infects epithelial cells of the intestine (Daszak, 1999). The pathogenic process starts during shizogonic phase of the parasite development. The pathogenic process during the first generation of schizonts is

negligible. However, the most pathological change is during the second generation of shizonts. Their development, deep in the cells of glands, results in inflammation, mucus desquamation, capillary rupture and hemorrhage (Chauhan, 2010). Cytokines and other factors derived from epithelial cells play important roles in inflammatory and immune responses in intestinal tissue. Once infected the intestinal epithelial cells are destroyed, or their function is impaired and, in more severe infections, an acute inflammatory reaction may be seen. The epithelium may become erosive or ulcerated resulting in hemorrhage in the lamina propria with hypoproteinemia and anemia being the consequences (Julie, 1999**)**. Death is a consequence of hemorrhage (bird can lose 60 to 80 percent of the blood volume), toxemia or as a consequence of gangrene or rupture of the intestinal wall (Ruff, 1991).

The coccidian species involved, prevailing environmental conditions, the size of the infective dose, the age of the host, the number of host cells destroyed, the location of the parasite in the tissues and the presence or absence of acquired immunity all will play a role in the pathogenesis. Pathology and clinical signs of coccidiosis are nearly always caused by the development of the gamont and oocyst in the lower ileum, caecum, and colon. Coccidiosis often leads to disturbances in lowering nutrient absorptions and ions and osmotic imbalance of the gut epithelium **(**Fitz-Coy and Edgar, 1992). Oocysts infect the cells of intestinal lining, replicate and cause them to burst, change the gut morphology, reducing gut length and truncating the intestinal villi. Pathology is largely associated with destruction of the epithelial lining of the infected part or intestine which results in reduced ability for the digestion and absorption of nutrient by the bird **(**Long *et al*., 1980).

2.5. Clinical findings

Clinical signs caused by infection with these parasites are referred to as coccidiosis, but their presence without disease is called coccidiasis. Clinical signs of coccidiosis are due to destruction of the intestinal epithelium and frequently, the underlying connective tissue of the mucosa (Safari, 2001). The first and most frequent symptom is at the beginning

yellow diarrhea. Signs may include discharge of blood, dehydration, severe diarrhea and high mortality (Chauhan, 2010). As the disease progresses, because of the blood in feces, feces are red or resemble the color of chocolate. The feathers around the cloacae are covered with bloody deposits (Calnek, 1997). Symptoms of the disease start to appear at the time when the second generation of schizonts to replicate, grow, mature and release the second generation of merozoits. Second generation of merozoits cause inflammation of the sub-epithelial mucosa, desquamation of the epithelia and capillary rupture in the intestinal wall. As a consequence, bloody diarrhea occurs (Jordan, 1990; Ruff, 1991). Death usually occurs 5 and 6 day after infection. It is postulated that death is the result of blood loss, as well as infection. Death of the bird can be the result of gangrene or rupture of the cecal sac (Calnek, 1997).

2.6. Diagnosis

2.6.1. Detection of Oocyst in feces

The finding of a few Oocysts by microscopic examination of smears from the intestine indicates the presence of infection, but not a diagnosis of clinical coccidiosis. Direct microscopic examination of intestinal mucosa can be used to find the intracellular and extracellular stages of coccidian (Anders and Jorgen, 1998). A small amount of mucosal scraping should be diluted with saline on a slide and then covered with a cover slip. Developing schizont, gametocytes, and Oocysts of Coccidia may be seen in smears taken from the suspected lesion, but mostly the lesion is caused by mature schizonts **(**Chauhan, 2010). Diagnostic characteristics which are of value include the clusters of the large schizonts of *E. necatrix* and *E. tenella,* the small round Oocysts of *E. mitis,* or the large gametocytes of *E. maxima.* Presence of clusters of large schizonts in the midgut area is pathognomonic for *E. necatrix,* and in the caecum indicates *E. tenella.* Oocysts associated with lesions in the duodenum are *E. acervulina, E. mivati,* or *E. praecox*, and Oocysts in the lower gut are *E. mitis, E. mivati* and *E. brunetti*. The combination of oocyst size, location in the gut, and appearance of the lesions gives considerable confidence in diagnosis (Graat *et al.*, 1996; Anders and Jorgen, 1998).

2.6.2. Post mortem examinations

When large numbers of Oocysts are found in the feces of live birds concurrent with diarrhea, emaciation, and pallor or pale skin color, coccidiosis should be suspected as the cause of illness. However, a diagnosis of coccidiosis as cause of death requires a necropsy evaluation combined with identification of the causative coccidian (Simon, 2005).

Gross lesions are distributed throughout the length of the intestine, predominantly in the middle portion of the small intestine (Magner, 1991). Lesions produced by *E. acervulina* and *E. mivati* occur primarily in the duodenal loop and the upper part of the jejunum. *E. maxima* and *E.necatrix* produce their most severe lesions in the mid-intestinal area, which is readily identified by the residual yolk sac diverticulum. *E. brunetti* invades the mucosa of the lower intestine and the rectum. Lesions of *E. tenella* are found mostly in the caecum, but occasionally some strains of *E. tenella* will cause lesions in the rectal area. The cecal wall is often greatly thickened because of edema and later scar tissue. The serosa of the unopened ceca shows the petechiae as coalesced and eroding the entire surface (Ghittur, 1997; Kennedy, 2001).The characteristics of the observed lesions such as its location on the intestinal tract, its appearance and severity, the nature of intestinal contents and other associated gross change can be useful in establishing a diagnosis (Conway and McKenzie, 2007; Fred *et al*., 2008).

Focal lesions of the intestinal epithelium and small necrotic foci in the sub-epithelial connective tissue resulted due to the first generation schizont maturation. On the cecums are enlarged in diameter and there are regions with petechiae in the mucosa. Entrance of the second generation of merozoits into the healthy epithelial cells, mark the moment when hemorrhage of the caecum start (Calnek, 1997). The intestinal content is watery, bright red in color with desquamated cells, erythrocytes and plenty of coccidian in different stages of development (Panda *et al*., 1997). The severity of lesions is roughly proportional to the number of Oocysts ingested by the bird and correlates with other parameters such as reduced weight gain, loss of skin pigmentation and diarrhea. In the

field, lesion scoring is generally useful in gauging the severity of infections but may not correlate with microscopic scoring (Conway and McKenzie, 2007).

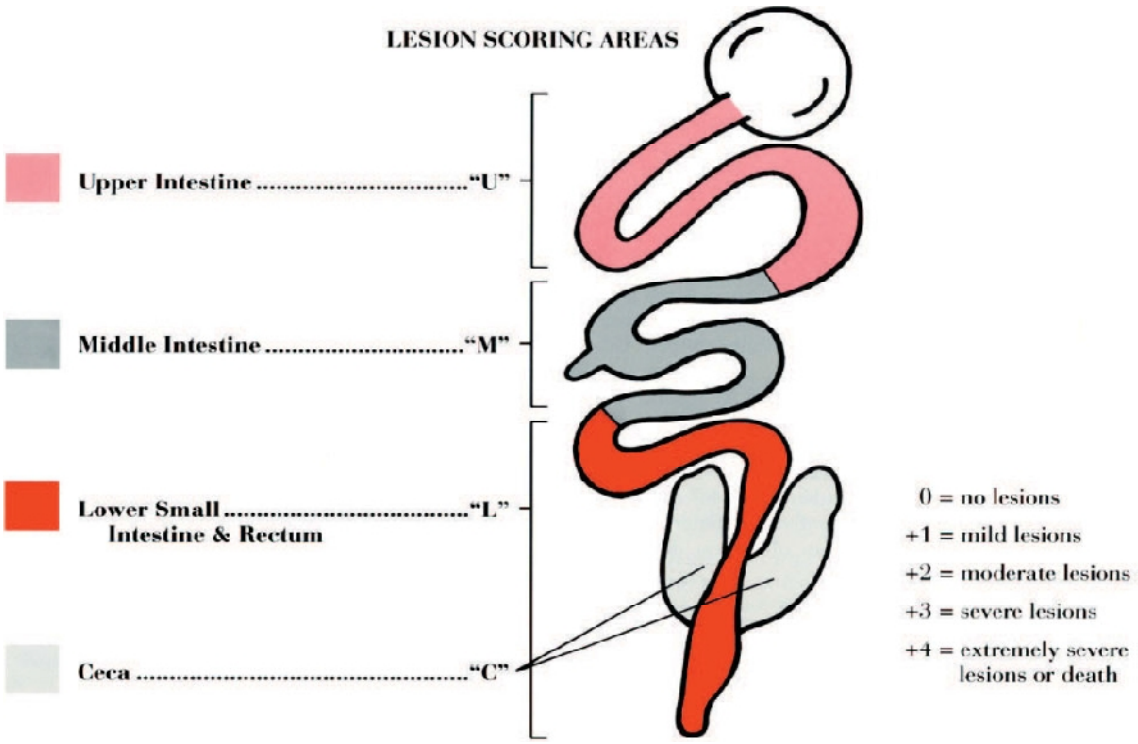

Figure 2: Lesion scoring areas (Conway and McKenzie, 2007).

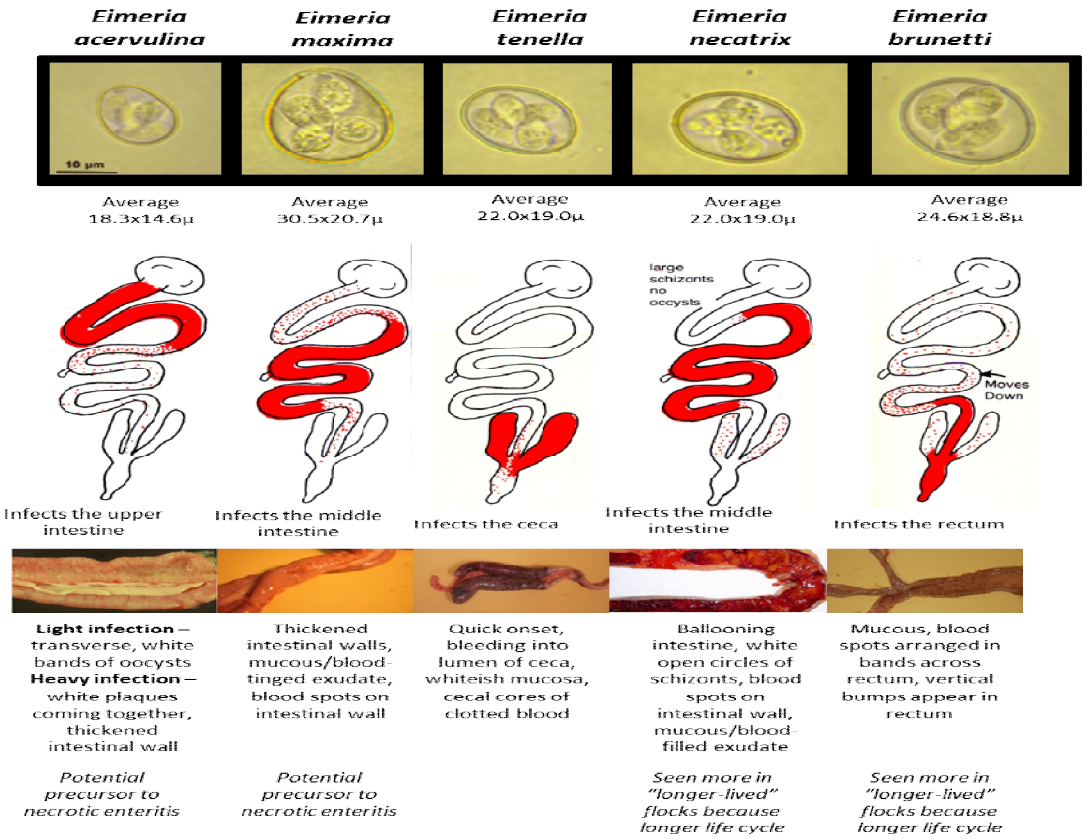

Figure 3: *Eimeria* Oocysts, location of infection along the intestine and lesions per species (Williams, 1999).

2.6.3. Histopathology

The wall of the gut is thickened indicating retention of fluid (edema). The lumen is enlarged two to three times. Surface of the epithelium, as well as the epithelium of the crypts are desquamated with patches of hemorrhage. There may be blood in the lumen of the gut indicating blood loss (hemorrhage), or merely retention of an excessive amount of blood in the tissue (hyperemia) there is also infiltration with various body reaction and the development of immune response (Marquardt *et al*., 2000).

Small focal areas of hemorrhage and necrosis may appear near blood vessels of the muscularis layer. Heterophil infiltration of the sub mucosa proceeds rapidly as the large

second-generation schizonts develop in the lamina propria. These are found in clusters or colonies that generally are progeny of a single first generation schizont. Maturation of the second-generation schizont is accompanied by excessive tissue damage, bleeding, disruption of the cecal glands, and destruction of the mucosa and muscularis layer. The sub mucosa becomes densely fibrotic. Microscopic examination of the intestinal wall reveals plenty of parasites in different stages of maturation and development with heterophil infiltration of the lamina propria and sub mucosa. The pathognomonic finding is the presence of schizonts in the tissue (Calnek, 1997).

2.6.4. Hemagglutination inhibition assays

The carbohydrates present on sporozoites and lectin-binding sites on the surface of sporozoites can be detected by means of peroxidase-conjugated lectins. Using hemagglutination inhibition assays, different stages of the parasites having specific surface sugar lectins of *E. tenella*, *E. acervulina* and *E. maxima* can be detected. The lectins found on the surface of the sporozoites play a role in determining the site of infection within the intestine of the host (Yoshitaka *et al*., 1999**)**.

2.6.5. Enzyme-linked immunosorbent assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) was adapted to detect antibodies to *Eimeria* spp. It is simple test and reliable methods for the determination of the exposure status of chickens to *Eimeria* species and detecting specific IgG and IgM antibodies in serum samples. The indirect ELISA assay may be possible to discriminate between chickens actually infected with *Eimeria* species (as indicated by high levels of ant-parasite IgM) and unexposed birds. The applicability of this ELISA, using sporozoites antigen of *E. tenella* to practical situations was substantially confirmed **(**Smith *et al*., 1993**)**.

ELISA analyses of serum pools having varying protective capacities revealed good correlations between passive protection and levels of anti-unsporulated oocyst, anti-sporulated oocyst, anti-merozoite and anti-gametocyte antibodies**.** The ELISA test is an initial comparison revealed few differences in their ability to monitor the onset, kinetics and magnitude of the antibody response. The merozoite antigen is selected for further evaluation because it was easier to prepare. The ELISA should prove useful for monitoring infectivity in vaccination programs in layer and breeder flocks and for assessing the effectiveness of bio-safety measures in broiler flocks (Constantinoiu *et al*., 2007).

2.6.6. Polymerase chain reaction (PCR)

A polymerase chain reaction (PCR) assay, based on the amplification of internal transcribed spacer regions of ribosomal DNA, was developed for the chicken coccidian species-specific primers for the detection and discrimination of all *Eimeria* species that infect the domestic fowl is now available. The PCR assay provided a faster, more simplified read-out compared to staining of amplified bands in an agarose gel with ethidium bromide (Beate *et al*., 1999**)**. For identification of *Eimeria species* and variations in genomic DNA two primers corresponding to highly conserved regions of the 18S ribosomal DNA of the coccidian forward primer and the reverse primer were chosen. The internal transcribed spacer 1 (ITS-1) from within ribosomal DNA (rDNA) genes was investigated to differentiate chicken intestinal coccidian to the genus and species level. The spacer separates the 3 end of the 18S ribosomal RNA gene from the 5 end of the 5.8S rRNA gene within individual rDNA transcription units. The cross-reaction would occur with the DNA from chicken intestinal contents and muscles, and the detection limit of PCR tested with pureline Oocysts of *E. tenella* **(**Yoshitaka *et al*., 1999; Andrew and Fang, 2003).

Several PCR based assays targeting different regions of the Eimeria genome have been described, such as the 5S rRNA, the small subunit rRNA, the sporozoites antigen gene EASZ240/160. Nevertheless, the practical implementation of these methods in routine

diagnostics and epidemiological studies of chicken coccidiosis has been limited, and the assays must still be regarded as experimental (Anita *et al*., 2007).

2.7. Economic importance of poultry coccidiosis

Coccidiosis remains one of the major disease problems of poultry in spite of advances made in prevention and control through chemotherapy, management and nutrition (Gari *et al*., 2008; Mersha *et al*., 2009). The disease causes high mortality, morbidity and adverse effects on the growth of infected birds. The incidence of coccidiosis in commercial poultry has increased due to higher stocking densities and intensive husbandry practices. The cost of anti-coccidial feed additives and treatment is estimated to exceed 400 million US$ annually in all poultry producing areas of the world (Ruzica *et al*., 2005). A great economic problem is due to primary of resistance to anti-coccidial drugs. Such drugs are not easy to use. Also, development of new drug generations, that are for prophylaxis and therapy, is expensive (Grag *et al*, 1999).

Poultry meat consumption, at a global level is constantly rising. So, there is a need to intensify broiler production. In such a production system, the possibility for coccidiosis is higher in spite of using anti-coccidial in feed. World trends in food production are to produce organic meat, with no drugs added to the feed. This means that the risk of coccidiosis is higher. Nevertheless, strategies to control coccidiosis are still based on prophylactic medication through feed and vaccination (Vermeulen *et al.*, 2001), not to exclude good production practices and good hygiene and sanitation.

2.8. Status of coccidioisis in chickens in Ethiopia

Table 1: Summary of coccidiosis in different areas of Ethiopia

Site of study	Prevalence	References
Debre zeit	71.7%	Ayana and Hailu, (2012)
Kombolcha	25.24%	Abdi *et al.*,2012
Addis Ababa	23.1%	Alemayehu *et al*. (2012)
Ambo	20.57%	Oljira *et al*. (2012)
Ambo	18.7%	Shiferaw, (2014)
Arsi Tiyo District	64.4%	Gari *et al*. (2008)
Central Ethiopia	25.8%	Ashenafi *et al*. (2004)
Tigray	17.5%	Yohannes *et al*. (2014)
Gomma wereda(Jimma)	11.69%	Meseret, 2010

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in poultry farms and backyard chicken in and around Ambo town, West Shewa zone of Oromia regional State from November, 2014 to April, 2015. Ambo town is the administrative center of West Shewa zone and located at 114 km away from Addis Ababa at 8°47'-9°20' North latitude and 37°32'-38°3' East longitude. The altitude of the area ranges from 1900-2101 meters above sea level. Ambo wereda has 34 administrative kebeles (ATMA, 2010).

The agro-ecology of the study area is 23% highland, 60% midland, and 17% lowland. The study area receives a mean annual rainfall of 900mm (800 – 1000 mm) and annual temperature ranging from 15^0c to 29^0c with average temperature of 24.5^0c. The area receives a bimodal rainfall with mean annual rainfall of 1225mm, in which the long rainy season extends from June to September, while the short rainy season occurs from March to May (ATMA, 2010).

The chickens population of the district is 105794. Both local and exotic poultry breeds are available in and around Ambo town kept under backyard and semi-intensive husbandry system (AARDB, 2006).

Figure 4: Map of study area (Etefa and Dibaba, 2011)

3.2. Study population

The study animals were Potchefstroom Koekoek (PK), Isa Brown (IB) and local breed (LB) of chickens in poultry farm of Ambo University and backyard chickens in and around Ambo town.

3.3. Study design and sample size

A cross sectional study design was conducted on those chickens that showed clinical signs indicative of coccidiosis to determine the hematological and pathological changes in chickens due to coccidiosis. A total of 113 chickens (25 IB, 36 PK and 52 local breed) were purposively selected from poultry farm of Ambo University and local farmers managed under traditional husbandry system in the study area. Local chickens were bought directly from the markets of study area. Chickens of both age and sexes were included proportionally.

3.4. Study methodology and data collection

3.4.1. Clinical examination

Purposively selected sick or suspected birds were closely examined for clinical manifestations suggestive of coccidiosis and clinical signs were recorded. After clinical examination the chickens were then transported to the Ambo university department of Veterinary laboratory technology laboratory for hematological, post-mortem and parasitological examinations.

3.4.2. Blood count determinations

Blood samples were collected from the brachial vein of 22 chickens from each breed (9 PK, 7 IB and 6 LB) that were prominently ill of bloody diarrhea using a 3ml sterile syringe and a 23-gauge needle. Each blood sample was transferred immediately into a sterile tube containing the anticoagulant EDTA. Total Red Blood Counting and Total White Blood Counting were determined by a manual method using haemocytometer (Campbell, 2007). The total RBC counts were performed in a 1:200 dilution of blood in normal saline and total WBC counts were performed in a 1:20 dilution of blood in 1% acetic acid solution. The RBCs were counted in four corners and one central squares of haemocytometer (Neubauers) chambers designed for RBC counting, and the numbers of

cells counted were multiplied by 1x 10^4 to estimate the total number of cells per cubic millimeter. The total WBCs were counted in four large corners square of haemocytometer (Neubauers) chambers designed for WBC counting, and the numbers of cells counted were multiplied by 50 to estimate the total number of cells per cubic millimeter as described by Coles (1986).

The Hemoglobin concentration (Hb) was evaluated by matching acid hematin solution against a standard color solution found in Sahl's hemoglobinometer. PCV was measured by a standard manual technique after centrifugation of 7mm microhematocrit capillary tubes filled with blood (Irizaary-Rovira, 2004). Erythrocyte indices (MCV, MCH and MCHC) were calculated from TRBC, PCV and Hb as reported by Campbell (2007).

3.4.3. Postmortem examination and lesion scoring

Post-mortem examination was conducted on the clinically sick and recently dead chickens suspected of coccidiosis for the presence of gross lesions consistent to the diseases following the procedure described by Conway and McKenzie (2007) and the gastro intestinal tract was thoroughly examined for gross pathological changes. From a total of 113 chickens examined, portions of intestinal and cecums with gross lesion were sampled from 26 chickens for further histopathological examinations.

Intestinal gross lesions in any part of the sections were graded from 0 to 4 based on the criteria described by Conway and McKenzie (2007). The lesion score zero represents absence of lesion and lesion score four represents very severe intestinal /caecum mucosa lesion and fatal cases. The color, shape and consistency of the lesions found on the intestines and caecum were also recorded.

3.4.4. Eimeria species identification and techniques used

Mucosal scrapings were taken from lesions of the upper (duodenum), middle(jejunum and ileum) intestine, large intestines and caecum in separate Petri-dish and placed on

microscopic slides, diluted with drop of tap water and examined under light microscope for the presence of oocyst according to the procedure utilized by Idris (2007).

Positive samples were further examined for species identification. *Eimeria* species were identified depending on the morphology (shape and size) by measuring oocyst size using a calibrated micrometer at 40x magnification, sporulation time, and color of the oocyst. Location and characteristics of intestinal lesion and histopathological finding were also used in species identification as described by Conway and McKenzie (2007). Each species of *Eimeria* was spread out in shallow Petri dish with 2.5% potassium dichromate solution for sporulation. The sporulation time was considered when 90% of the Oocysts were sporulated (Conway and McKenzie, 2007).

3.4.5. Histopathological examination and lesion scoring

The presences of gross lesions were carefully recorded and 3-5 mm thick tissues with lesion were collected from intestines and caecum. The tissue were immediately fixed in 10% neutral buffered formalin and submitted to NAHDIC, Sebeta, Ethiopia. The fixed tissues were trimmed and processed in an automatic tissue processor. Briefly the tissues were dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin, sectioned at 5 µm, stained using haematoxylin and eosin (H and E) and mounted with distrene plasticizer xylene (DPX) (Bancraft *et al.,* 1990). The slides were examined under microscope for histopathological characterization of lesions.

Severity of microscopic pathological lesions was graded(Annex IV) as very severe (+4) that indicates numerous parasite developmental stage, severe hyperplasia of tunica muscularis, congestion, massive lymphocyte, heterophil and macrophage infiltration, coagulation necrosis and sloughing off luminal epithelia. When large number of parasite, with lymphocyte infiltration in the tissue, congestion, necrosis and sloughing off luminal epithelia was observed the lesion was graded as +3. When moderate number of parasites with moderate congestion, lymphocyte infiltration, and necrosis was observed the lesion

was graded as +2. The mild (+1) lesion was indicated by mild presence of parasite, and mild lymphocyte infiltration in the tissue (Williams, 2005).

3.5. Data management and analysis

The data collected were coded and entered into Microsoft Excel and descriptive statistics were utilized to summarize the data using Software program for Social Science (SPSS) version 20. Percentage and tables were used for analyzing data of each hematological parameter. All hematological values were expressed as mean ± standard deviation. ANOVA test was used to assess if there was a statistically significant between hematological variables with categorical variables. In all cases, a 95% CI was employed to estimate sample results to the target population in the study area. Values of $P < 0.05$ were considered statistically significant.

4. RESULTS

4.1. Clinical signs

Clinical study on suspected chicken revealed that bloody diarrhea, depression, weakness, anorexia, ruffled feathers, weight loss, and death, which are suggestive of acute coccidiosis (figure 5).

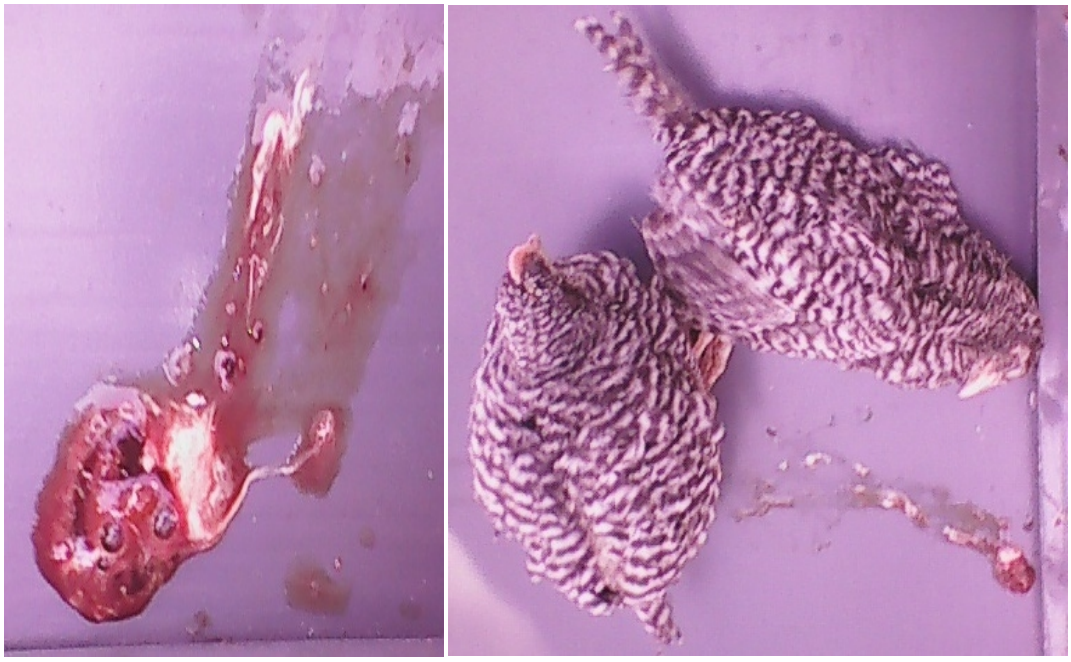

Figure 5: Bloody diarrhea (left), depression and ruffled feathers (right) from koekoek breed that were observed during clinical examination.

4.2. Isolation and identification of *Eimeria* species

Eimeria species were determined based on the Oocyst measurement, site in the intestine and sporulation time. Based on this five Eimeria species, namely *E. acervulina*, *E. necatrix*, *E. brunetti*, *E. tenella* and *E. maxima* were identified in single infections. Mixed infections by *E. tenella* in combination with any one of the four species (*E. brunetti*, *E.*

necatrix, *E. acervulina* and with *E. maxima*) were common findings (Table 2). *E. tenella* was the most frequently isolated species (41.8%) followed by *E. necatrix* (11.9%), *E. brunetti* (10.4%), *E. maxima (6%) and E. acervulina* (3%).

E. tenella and *E. brunetti* were identified from caecal and lower intestinal mucosa, respectively, of chickens suffering from bloody coccidiosis. *E. tenella* was identified easily by its predilection site (caecum), characteristic lesions (bleeding), ovoid oocyst, oocyst size (22 x 18 μm) and clusters of large schizonts in the caecum. *E. brunetti* was identified by its location, lesion found at the lower large intestine, the presence of petechial hemorrhages, ovoid oocysts and oocysts size (25 x20 μm). *E. maxima* was the largest in size (28 x 24 μm), ovoid in shape and has golden brown color observed during microscopic examinations of mucosal scraping smears taken from the middle part of small intestine. *E. acervulina* was the smallest in size (16 x 14 μm) and ovoid in shape usually found in the duodenal loop showing white spots from the serosal side and mucoid exudates in intestinal content. *E. necatrix* was identified by its locations (middle intestine), oblong ovoid oocyst, oocyst size (20 x 17 μm) and the presence of severe bleeding and whitish plaques in the middle intestine on both sides of yolk sac diverticulum.

Table 2: Frequency distribution of Eimeria species isolated as single and mixed infections.

Eimeria species as single & mixed	Frequency of Isolation (%)	№ positive from visible gross lesions	Histopathological results	
			№ examined for histopathology	№ sections showing characteristic (%)
E. tenella	28 (41.8%)	22	11	10(91%)
E. necatrix	8(11.9%)	8	3	2(66.7%)
E. brunetti	7 (10.4%)	5	3	2(66.7%)
E. maxima	4(6.0%)	3	2	1(50%)
E. acervulina	2 (3.0%)	2	1	1(100%)
E. tenella + E. brunetti	3 (4.5%)	3	1	1(100%)
E. tenella + E. necatrix	4 (6.0%)	4	2	2(100%)
E. tenella + E. maxima	5 (7.5%)	2	1	1(100%)
E. tenella + E. acervulina	2 (3.0%)	1	1	1(100%)
E. acervulina + E. brunetti	1 (1.5%)	1	0	-
E. maxima + E. brunetti	1(1.5%)	1	0	-
E. necatrix + E. brunetti	2 (3.0%)	1	1	1(100%)
Total	67 (100%)	53	26	22(84.6%)

4.3. Hematological changes

Those chickens showing bloody coccidiosis (*Eimeria tenella* and *Eimeria brunetti*) revealed a general reduction in total RBC (1.69 ± 0.12 million), PCV (20.6 ± 2.2 percent) and Hb (8.8 ± 2.3). Koekoek breeds showed higher reduction of hemoglobin than local and Isa brown breeds with statistically significant ($p< 0.05$) as indicated in table 3. Male chickens indicated higher reduction of both Hb and PCV than that of females in bloody coccidiosis although the difference was not statistically significant ($P > 0.05$) (table 4). Young chickens showed more reduction of PCV and Hb than adult but the difference was not statically significant ($P > 0.05$). The MCV, MCH and MCHC value were within normal reference ranges. Total WBC count (3.4 ± 0.34) was increased in all Eimeria positive chicken. However, there was difference in an increment of WBC at different breeds with higher WBC count for Isa brown breed and the difference was statistically significant ($p< 0.05$).

Table 3: Hematological parameters in chickens infected by bloody coccidiosis with breeds

	Breeds				
Blood parameters	PK (N= 9)	IB (N= 7)	LB (n= 6)	Total (N= 22)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	P-value
RBC(1×10^6 cells/µl)	1.67 ± .09	1.74 ±0 .12	1.69 ± 0.14	1.69 ± 0.12	.430
PCV %	20.4 ± 2.4	21.7 ± 1.4	19.5 ± 2.3	20.6 ± 2.2	.178
Hb (g/dl)	7.34± 2.9	9.8 ± 0.69	9.7 ± 1.5	8.8 ± 2.3	.046
Total WBC(1×10^4 /µl)	3.24 ± 0.37	3.6 ± 0.24	3.5 ± 0.23	3.4 ± 0.34	.049
MCV (fl)	123.6 ± 18.0	126.3 ± 11.2	115.3 ± 10.2	122.1 ± 14.3	.379
MCH (pg)	42.9 ± 16.3	41.9 ± 5.7	39.9 ± 5.9	41.8 ± 10.9	.885
MCHC (g/dl)	33.9 ± 9.6	33.3 ± 4.4	34.7 ± 5.4	33.9 ± 6.9	.937

PK= Potchefstroom Koekoek breed, IB=Isa brown, LB= Local breed,

SD= Standard deviation

Table 4: Hematological changes in chickens affected by bloody coccidiosis with age & sex

Sex				
Blood parameters	Male (N= 13)	Female(N= 9)	Total (N= 22)	
	Mean ± SD	Mean ± SD	Mean ± SD	P-value
RBC(1×10^6 cells/µl)	1.69 ± 0.21	1.71 ± 0.11	1.69 ± 0.12	.793
PCV %	20.3 ± 2.3	20.8 ± 2.1	20.6 ± 2.2	.652
Hb (g/dl)	8.2 ± 2.6	9.2 ± 2.1	8.8 ± 2.3	.326
Total WBC(1×10^4 /µl)	3.5 ± 0.30	3.4 ± 0.4	3.4 ± 0.34	.772
MCV (fl)	123.7 ± 12.9	119.7 ± 16.6	122.1 ± 14.3	.529
MCH (pg)	43.8 ± 10.1	38.8 ± 12.1	41.8 ± 10.9	.296
MCHC (g/dl)	35.4 ± 7.4	31.9 ± 5.9	33.9 ± 6.9	.245
Age				
Blood parameters	Young (N= 11)	Adult (N= 11)	Total (N= 22)	
	Mean ± SD	Mean ± SD	Mean ± SD	P- value
RBC(1×10^6cells/µl)	1.67 ± 0.7	1.72 ± 0.14	1.69 ± 0.1	.357
PCV %	20.1 ± 2.2	21.0 ± 2.0	20.6 ± 2.2	.386
Hb (g/dl)	8.3 ± 2.2	9.4 ± 2.4	8.8 ± 2.3	.279
Total WBC(1×10^4 /µl)	3.5 ± 0.45	3.4 ± 0.21	3.4 ± 0.34	.957
MCV (fl)	118.5 ± 13.5	125.6 ± 14.8	122.1 ± 14.3	.249
MCH (pg)	44.9 ± 12.8	38.6 ± 8.3	41.8 ± 10.9	.189
MCHC (g/dl)	35.5 ± 7.9	32.5 ± 5.6	33.9 ± 6.9	.329

4.4. Gross lesion characterization and scoring

From a total of 113 chickens examined, 67 (59.3%) had coccidiosis. Fifty three (79.1%) of the coccidia infected chickens, showed visible gross lesions in their intestine and/or caecum. However, 14 (26.9%) chickens didn't show visible gross lesions although coccidia were detected in their mucosal scraping. When lesion severity grade was compared by chicken breeds 52.8% (28/53), 30.2% (16/53) and 17% (9/53) of koekoek breeds, local and Isa brown breeds, respectively showed mild (+1) and above lesions in their intestine and/or caecum. The frequency of detection of gross lesion score (GLS) in koekoek breed was significantly higher than that of local and Isa brown chickens (χ^2 = 20.731, $P < 0.05$) as indicated in the table 5. Young chickens showed more severe lesions than adult but the difference was not statically significant (χ^2 = 4.33, $P > 0.05$). Similarly lesions score between male and female was not significantly different (χ^2 = 0.297, $P > 0.05$).

Table 5: Comparison of lesion grades in different variable

Variables		№ examined	№ positive for *Eimeria* species	№ chickens showed Visible gross lesions	χ^2 value	p-value
Age	Young	69	39(56.5%)	34(64.2%)	4.33	.115
	Adult	44	28(63.6%)	19(35.8%)		
Sex	Male	61	30(49.1%)	23(43.4%)	.297	.862
	Female	52	37(71.2%)	30(56.6%)		
Breed	Koekoek	36	29(80.6 %)	28(52.8%)	20.73	0.000
	Isa brown	25	13(52.0 %)	9(17.0%)		
	Local	52	25(48.1%)	16(30.2%)		
Total		113	67(59.3%)	53(100%)		

The predilection site and characteristic lesions produced by specific *Eimeria* species were used for identification of the species. Necropsy examinations revealed that those chickens positive to *E. tenella* showed cecal hemorrhage, distended caecal pouch filled with clotted blood and necrosis of the caecum. The serosa of the unopened caecum shows the coalesced petechiae and eroded surface (figure 6 and figure 7). Caecum content becomes hardened as the sloughed mucosal surface joins the bloody material to form a cecal core and mixed with fibrinous exudates and acquires a cheese like appearance (Figure 8).

Chicken positive for *E. necatrix* and *E. maxima* showed similar gross intestinal lesions with very few exceptions. Chicken positive for *E. necatrix* showed severe bleeding and whitish plaques in the middle intestine on both sides of yolk sac diverticulum which was not the case in *E. maxima.* The severity of intestinal lesions for chicken infected with for E. *necatrix* were graded as (+3) in those with diffused hemorrhage in the opened ileum and crowded on the serosal surface, numerous plaques like lesions, intestine mucoid blood filled exudates and as (+2) in those that showed petechial or multiple hemorrhages through the mucous of the jejunum (figure 9).

Eimeria maxima infections are located in the midintestinal area on either side of the small knob (rudimentary diverticulum) left by the yolk sac. Gross lesion of chicken infected with *E. maxima* showed multiple hemorrhages on the serosal surface of the intestine and ballooning of the middle intestinal wall (figure 10).

Eimeria acervulina usually occurred in the duodenal loop showed mucoid exudates in intestinal content, white spots from the serosal side and eroded mucosal membranes. The opened lower loop of the duodenum showed distinctive transversely elongated white plaques on the serosal and dull red spots (hemorrhages) on the mucosal surface (Figure 11).

Eimeria brunetti was observed in the lower intestine extending down into the large intestine (between the ceca) and rectum. Diffuse hemorrhagic streaks on the mucosa,

blood-tinged contents and coagulated material sloughed off and appears mixed with the cecal contents were observed (figure 12).

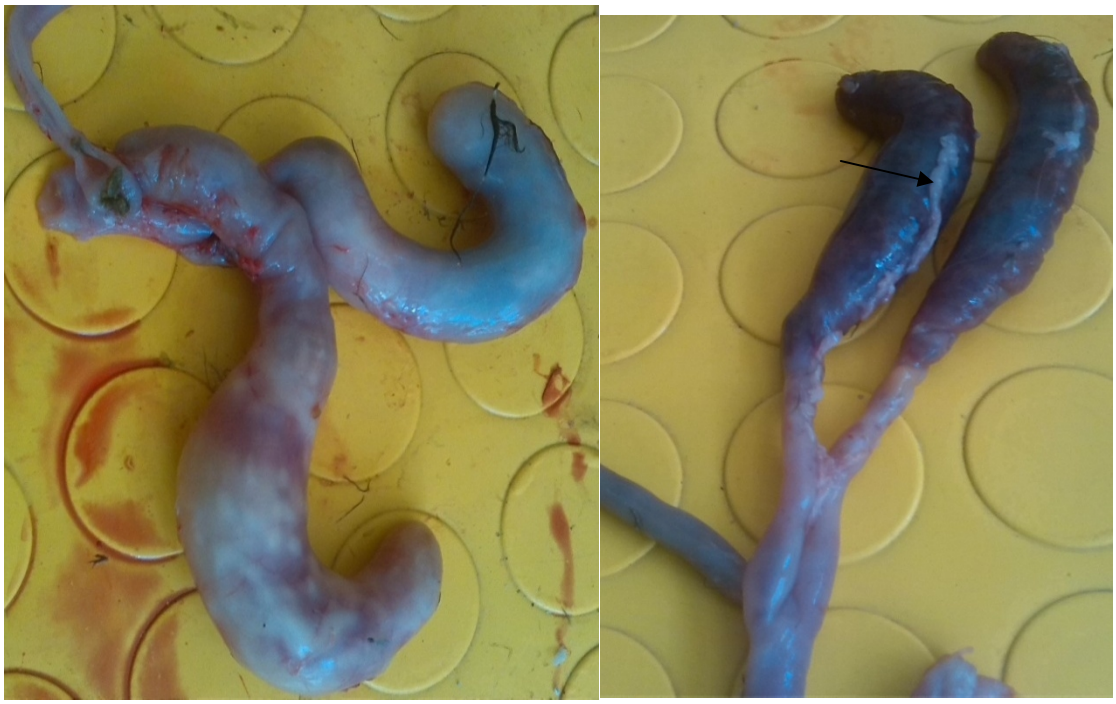

Figure 6: Caecal pouch greatly distended with clotted blood (right) & hemorrhagic serosa surface appears eroded (left arrow) in chicken positive for *E.tenella.* These lesions were graded as (+4).

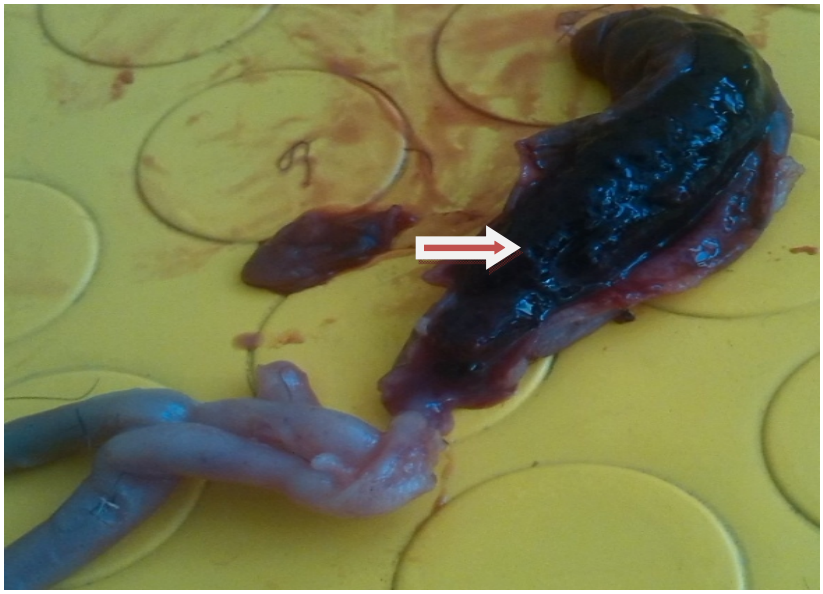

Figure 7: Severe hemorrhagic typhilitis with clotted blood in an opened caecum (arrow) from *E. tenella* positive chicken and this lesion was graded as (+4).

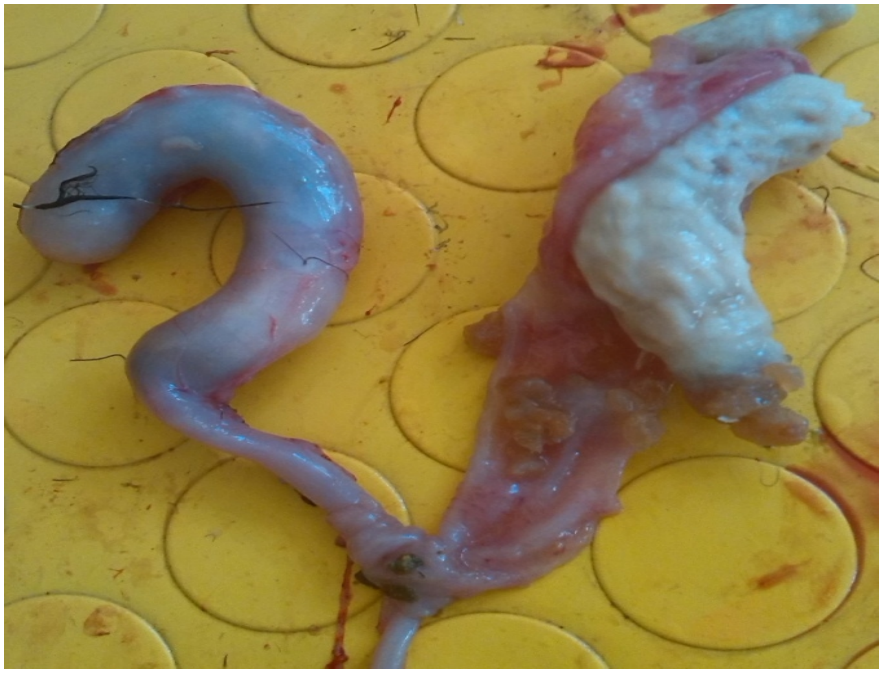

Figure 8: Caecum with cheesy material mixed with fibrinous exudates. On removal of the material the underneath caecum was severely hemorrhagic due to *E.tenella* & lesion was graded as +4.

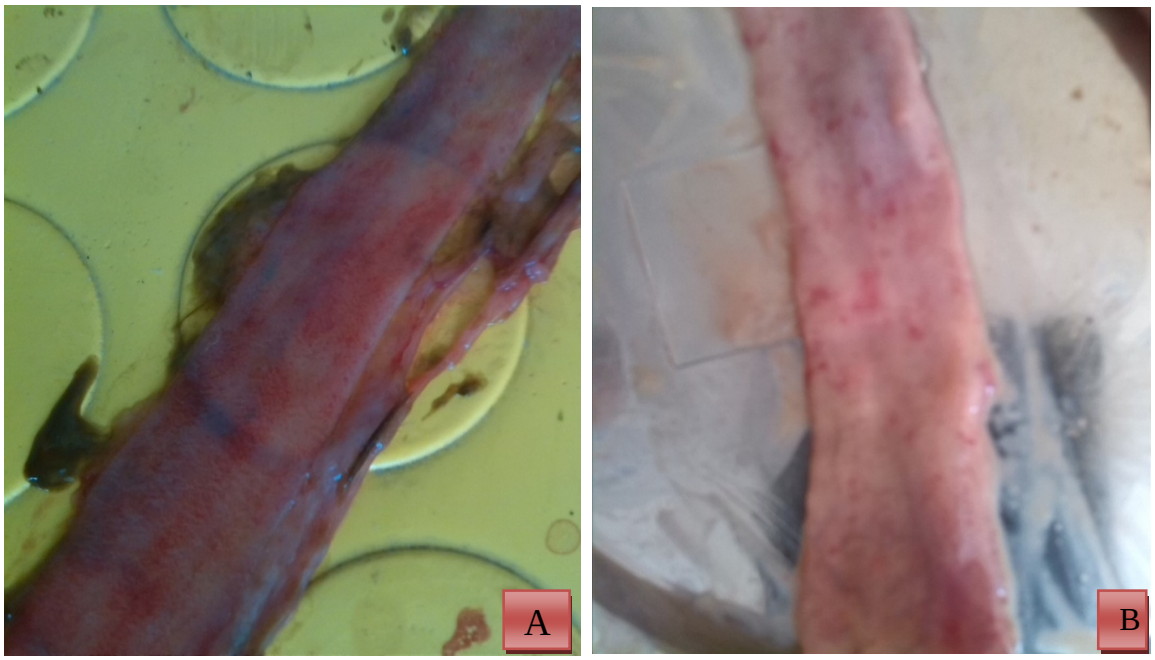

Figure 9: Ileum from Isa brown chickens with diffused hemorrhage (A) and jejunum with multiple petechial hemorrhages (B) (positive for *E.necatrix*).

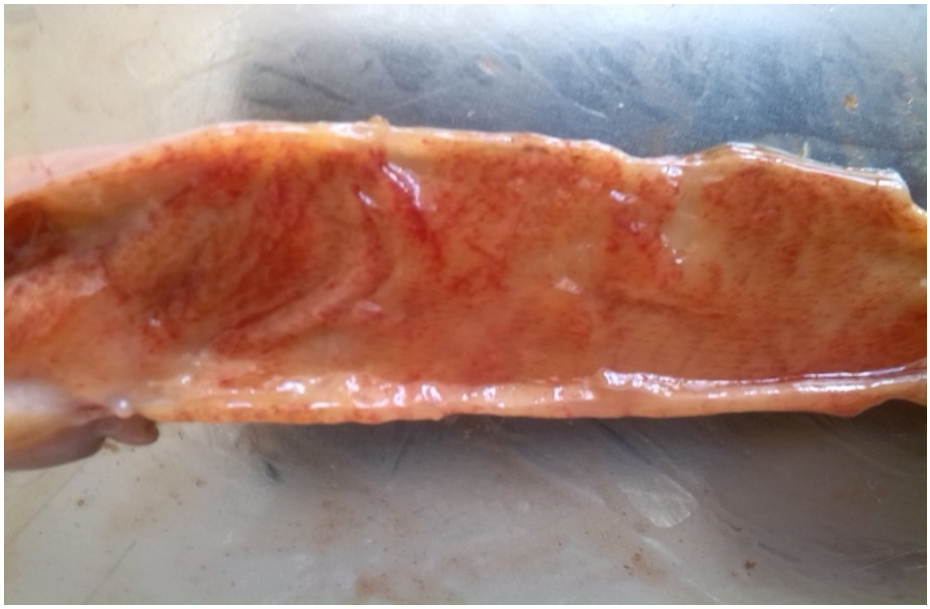

Figure 10: Ileum from local chickens that was positive for *E. maxima* with multiple hemorrhagic streaks and graded as +3.

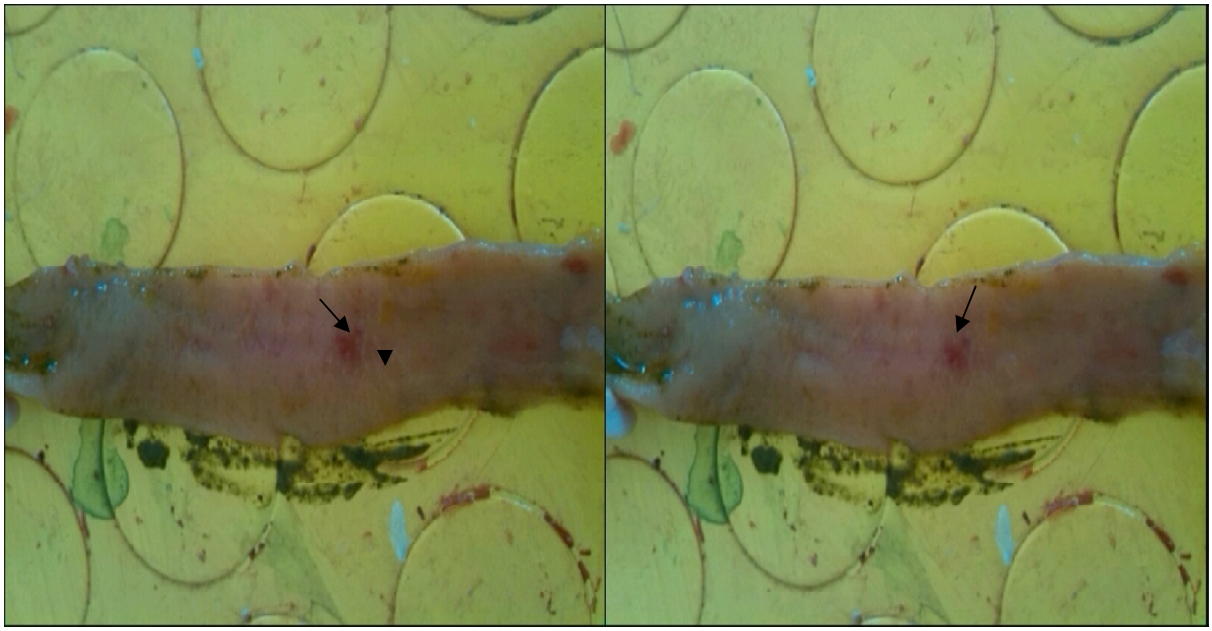

Figure 11**:** Duodenum with distinctive transversely white plaques on the mucosal surface (arrow head), petechial hemorrhages (arrow) positive for *E. acervulina* and graded as +2.

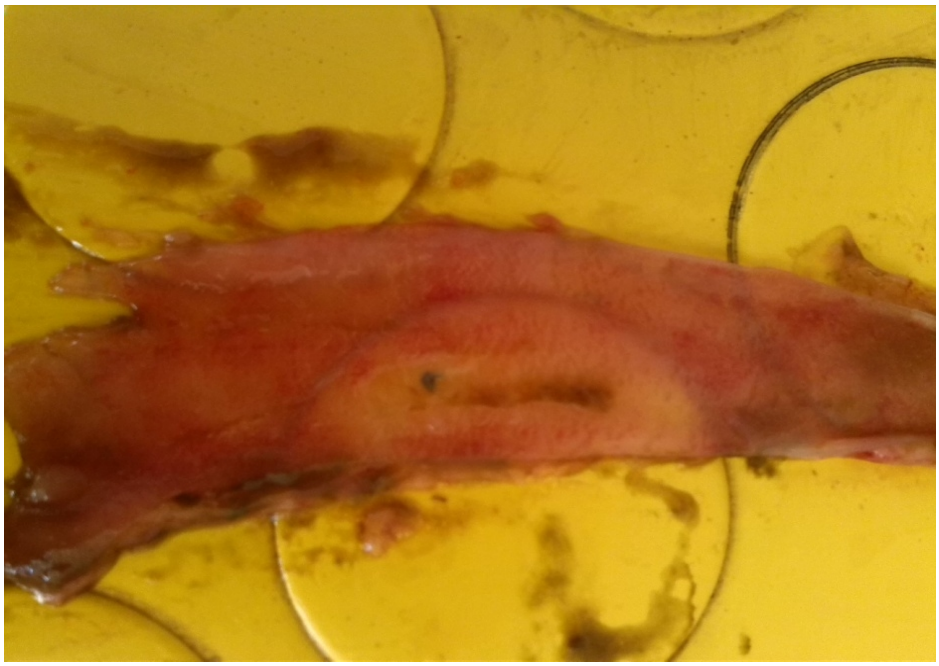

Figure 12**:** Large intestine of local chickens that was positive for *E. brunetti* with diffuse hemorrhagic streaks on the mucosa. It was graded as +3.

4.5. Microscopic lesion characterization and scoring

Histopathological examination of intestine and caecum of chicken that were positive for a single or mixed coccidia species generally showed numerous parasites invading the mucosal and sub-mucosal layers, desquamation and blunting of villi, hemorrhage, necrosis of the mucosal layer and infiltration of inflammatory cells into the sub-mucosa.

Chickens positive for *E. tenella* were characterized principally by complete desquamation of epithelium, dilation and necrosis of sub mucosal glands, multifocal areas of severe inflammatory infiltrate, discrete hemorrhage associated with various forms of the parasite (figure 13, 14 and figure 15). Cecal lesions were very severe at sites with massive accumulations of shizonts.

Chickens positive for *E. acervulina* showed lesions with moderate villous atrophy and fusion of villi, discrete hemorrhage, marked proliferation of epithelial cells of crypts, foci of intense mononuclear infiltrate in the sub-mucosa, multifocal and discrete interstitial edema at the junction of sub-mucosa and muscularis.

The middle small intestine of chicken positive for *E. necatrix* showed the characteristic coagulative necrosis, focal hemorrhages and deeply embedded in tunica mucosa and serosa. Sub-mucosa and lamina propria were invaded by large clusters of shizonts, large areas of the mucosa were sloughed off, and the lesion may go deep in to the muscularis layer and even to the serosal membrane (figure 16 and 17). Some epithelial cells may contain more than one parasite. Capillaries were engorged with red blood cells and there was infiltration of granulocytes in the parasite infected area (figure 17).

Lesions characteristic of *E. maxima* infection were discrete villous atrophy, proliferation of epithelial cells, cystic dilation of the sub-mucosa, numerous petechial hemorrhage, dilation of the sub-mucosal glands and various developmental stages of the

parasite. There were also severe muscular edema, necrosis of mucosa, sub-mucosa and presence of clusters of schizonts (figure 18).

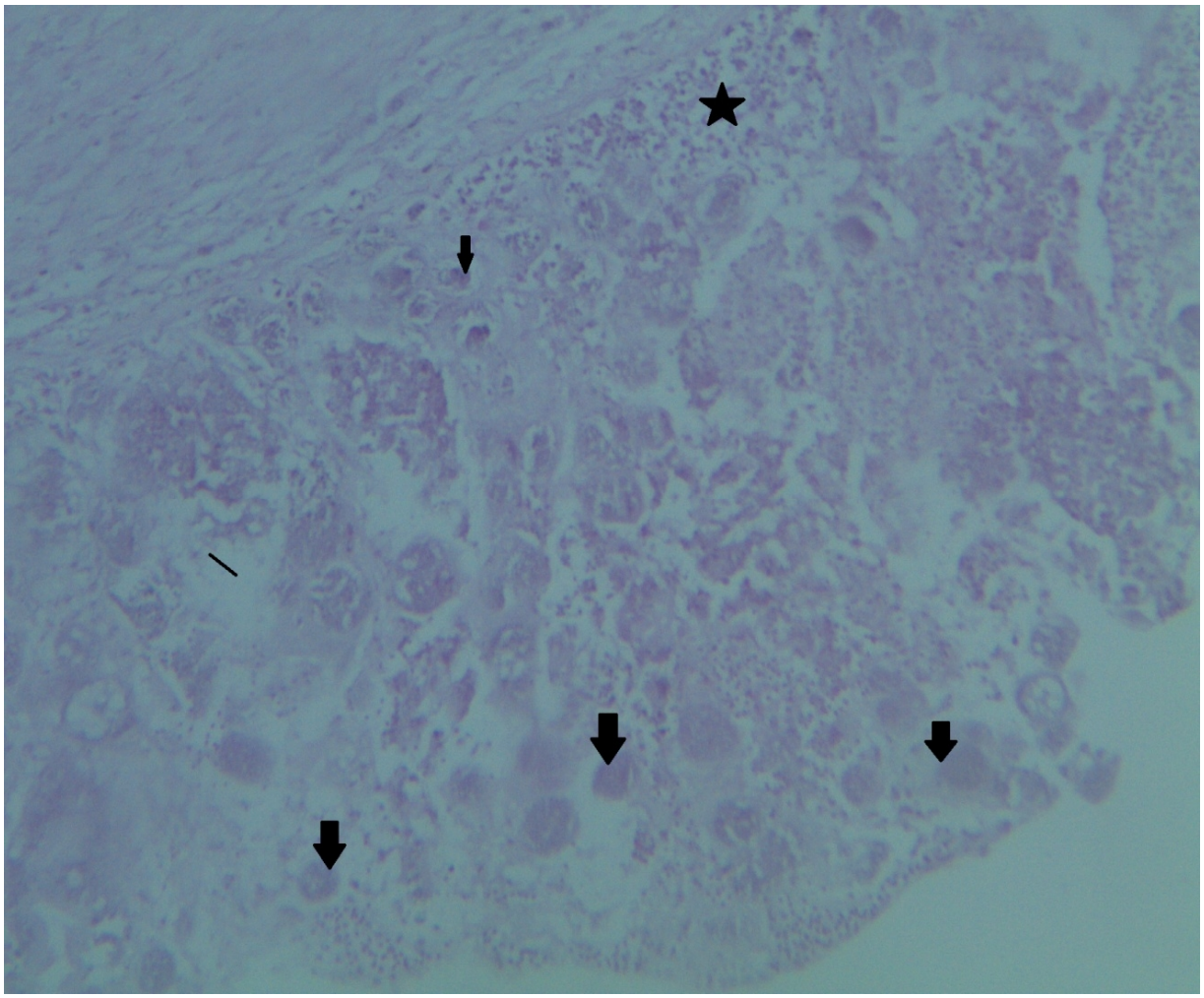

Figure 13: Caecum infected with *E.tenella* showing, complete desquamation of epithelium by parasite (arrow) necrosis of sub-mucosal glands (line) & diffused inflammatory cells infiltration (star) (scored as + 4) H &E stain 40x

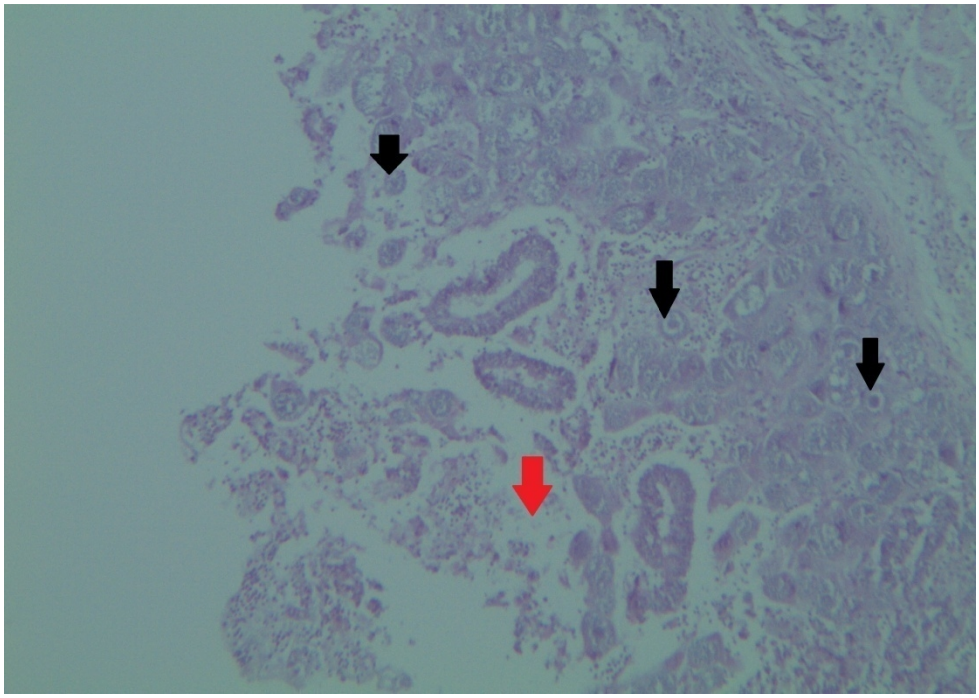

Figure 14: Cecum from *E.tenella* positive chicken showing severe erosion, loss of crypts (red arrow) & different developmental stages of the parasite (black arrow) (graded as +3) H& E stain 10x.

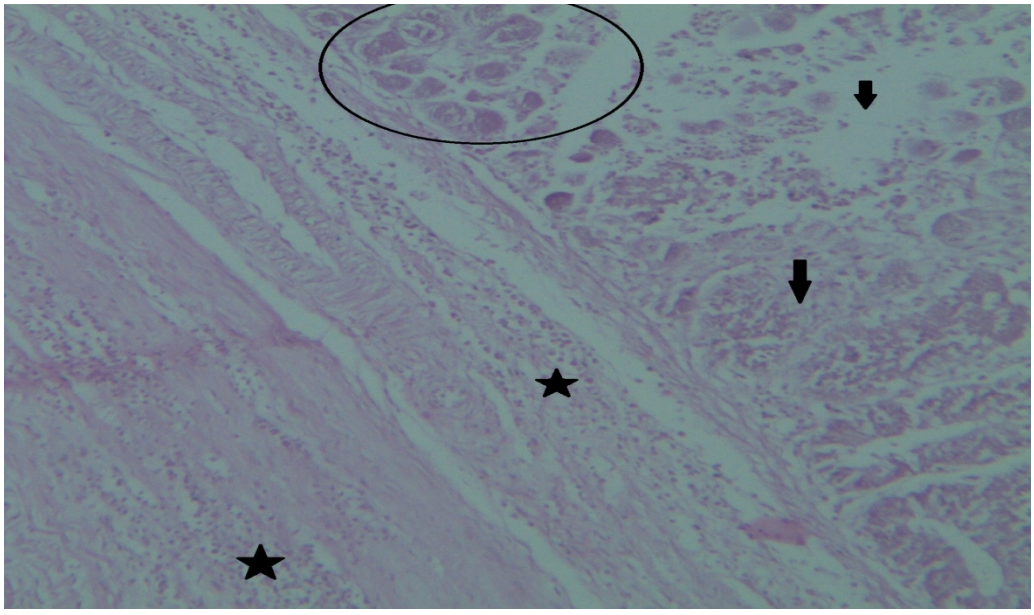

Figure 15: Chickens positive for *E.tenella* showing loss of crypt by parasite (circle), necroses of sub-mucosal gland (arrow) & inflammatory cells infiltrations in basal lamina and muscularis layer (stars) graded as +4 H & E stain 40x.

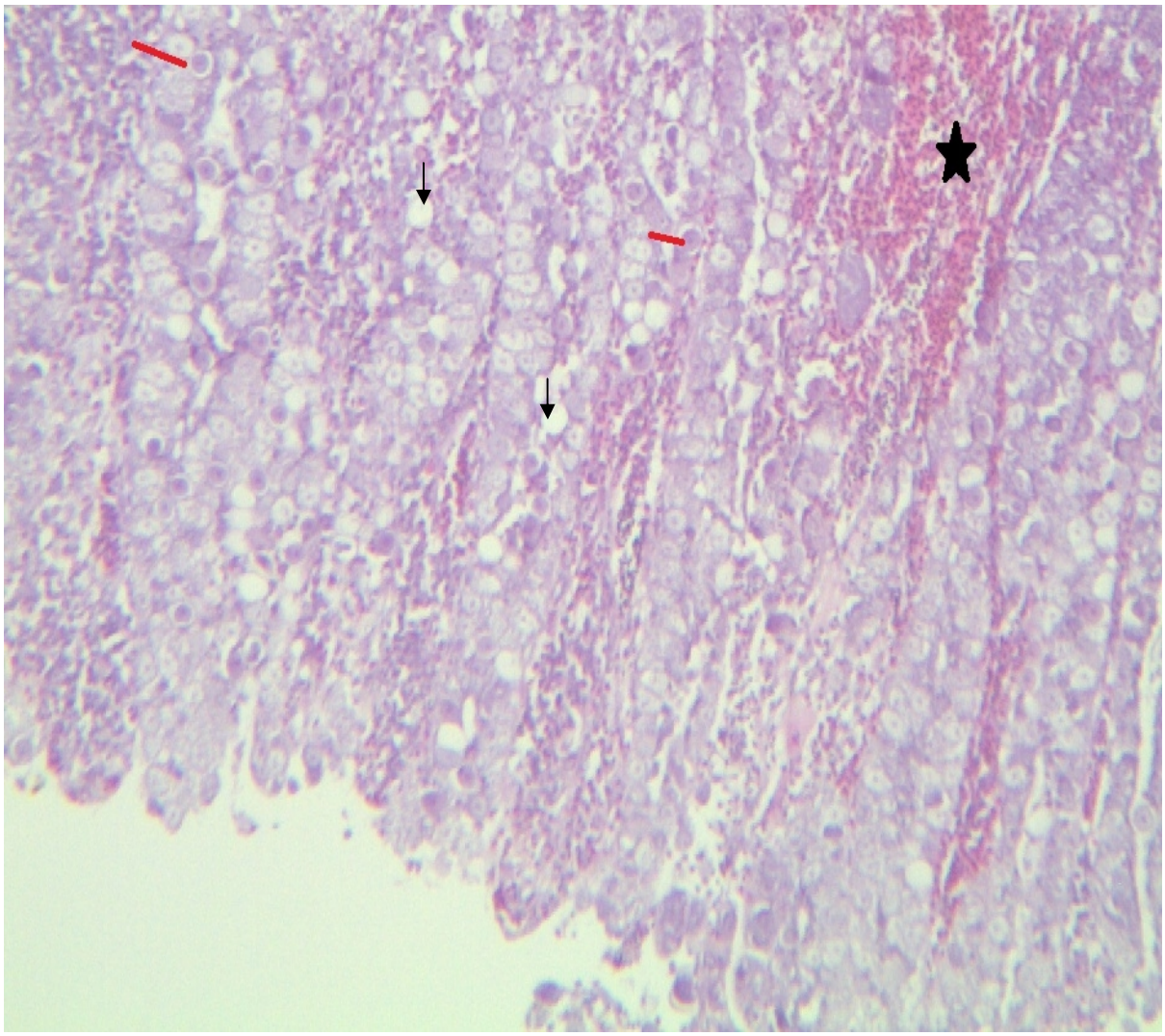

Figure 16: Jejunum from *E.necatrix* positive chicken (+3) with villous erosion & fusion, goblet cell hyperplasia (arrow) with different stages of parasite (lines) and Severe hemorrhages deep into the sub-mucosa (star) H & E stain 40x.

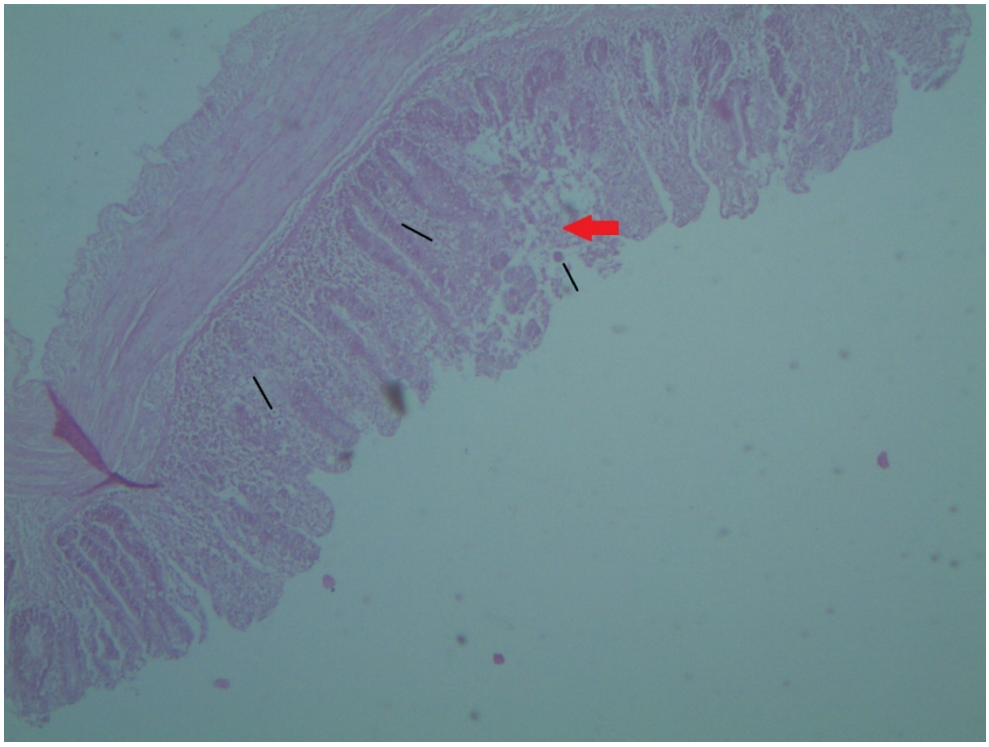

Figure 17: Jejunum from *E.necatrix* chicken with erosion of upper part of villi (red arrow) and different stage of parasite (lines) (graded as +3) H & E stain 10x.

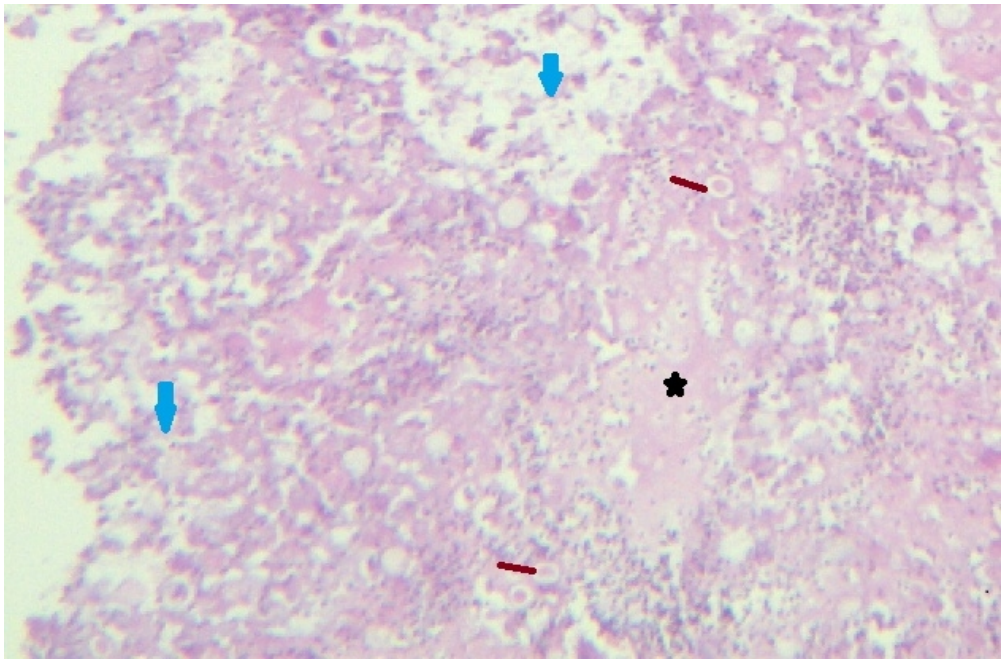

Figure 18: Ileum from *E.maxima* positive chicken with proteinous edema (stars), severe cellular necrosis (arrow) & parasite developmental stage (line) (graded as +4) H & E 10x.

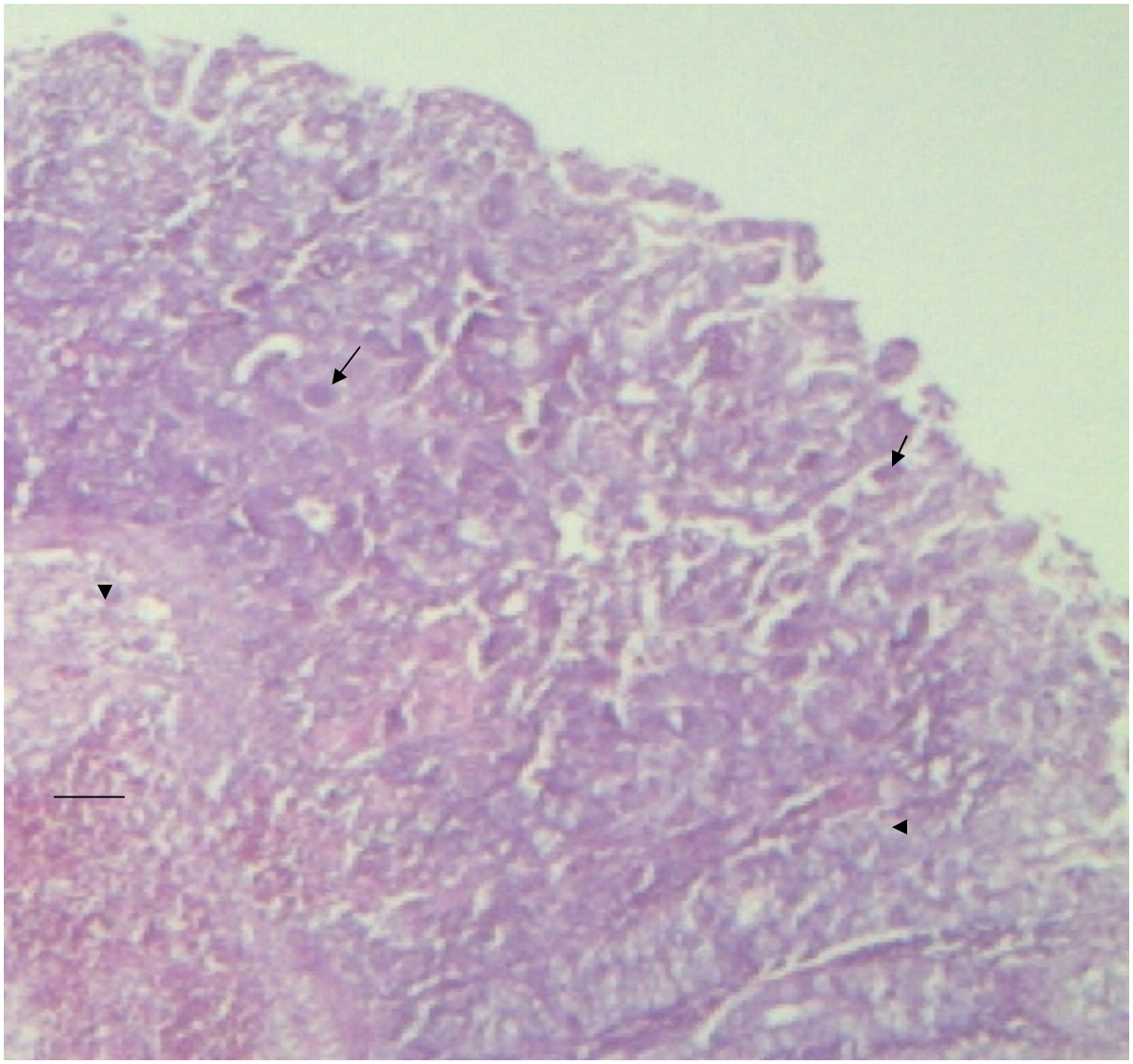

Figure 19: Large intestine from *E.brunetti* positive with necrosis (arrow head) & erosion of mucosal by the parasite (arrow) & severe hemorrhage in the sub-mucosal layer (line) (scored as +3) H & E stain 40x.

5. DISCUSSION

In the present study, caecum distended with bloody faeces and mucoid debris along with hemorrhages on the mucosa was observed. The findings of this characteristic feature of coccidiosis is in agreement to the report of Meskerem *et al.* (2013) who observed enlargement of caecum with clotted blood, hemorrhages throughout caecal mucosa, thickened caeca, shortened and consistent cases of necrosis and change in coloration from reddish to milky white in chickens positive for *E. tenella.*

Chicken with bloody coccidosis showed general reduction in total RBC, Hb & PCV. Wakenell (2010) and Meskerem *et al.* (2013) showed that chickens with bloody coccidosis showed a higher reduction in total RBC and PCV. Fukata *et al.* (1997) reported lower counts of total RBC and PCV in chickens infected with *E. tenella* and *E acervulina* when compared to the uninfected controls. Ogbe *et al.* (2010) also reported a slight drop in the PCV, Hb and RBC counts in *E. tenella* infected broilers. The reduction in the RBC and Hb could be due to the loss of blood into the gastrointestinal tract (external blood loss) (Irizaary-Rovira, 2004; Meskerem *et al*, .2013).

This study has shown that anemia for *Eimeria* positive chicken was normocytic normochromic (normal levels of MCV and MCHC). MCV and MCHC do not difference statistically between breed, age and sex ($p > 0.05$). There may be due to liberation of large quantities of histamine due to injury to the tissues which increase the permeability of capillaries and venues allowing exudation of large quantities of fluid. The present study also showed higher decreases in Hb and PCV of male chickens infected with bloody coccidosis than in females. These were similar to the report by Manal and Azza (2005) that Hb and PCV were influenced by androgen.

Total WBC (leukocyte) count showed an increased numbers when compared with the reference value indicated by Merck Veterinary Manual (2011) and statistically significant difference between breeds ($p < 0.05$). This was may be due to breed factors. The present results were in agreement to those reported by Rose *et al.* (1979) who indicated that the

peripheral blood leukocytes (PBL) response to infection with *E. maxima* and *E. acervulina* in chicken shows the increment in the number of PBL. In primary infections, the number of PBL increased and changes were found in the count of polymorphonuclear cells, lymphocytes and large mononuclear cells. The increase in WBC count might be due to induction of immune response in the infected birds due to increased lymphopoiesis as a first step of defense mechanism to infection. Similar findings were also mentioned by Ricklefs and Sheldon (2007) who found high count of WBC in parasitic (malaria and haemosporidin) infected birds.

In the present study, five *Eimeria* species were identified in naturally infected chickens in the study area. Positive chickens were harboring one or more *Eimeria* species. About 26.7% (18/67) of positive chickens were found to be infested with more than one Eimeria species while 73.3% (49/67) of the positive chickens were infested with single species. In the present study *E.tenella* was the predominant species, followed by other species. However, previous studies conducted in Ethiopia Safari (2001) and Ashenafi *et al.* (2004) revealed that *E. acervulina* was the most prevalent species. On the other hand Lobago *et al*. (2005) reported that *E. brunetti* was the most prevalent species. However, this result agreed partially with the result recorded by Abu Elezz (1994) who stated that, the cecal coccidiosis *E.tenella* is the most prevalent species in balady chicks in Egypt. Haug *et al.* (2008) confirmed that *E. tenella* and *E. maxima* were the most prevalent species associated with medium-sized and large oocysts, respectively in broiler chickens in Norway. The probable reasons for this discrepancy could be the difference in agro-ecology, virulence of the *Eimeria* species and different management system and/or due to other possibility like drug resistance.

Eimeria tenella and *E. brunetti* were previously reported from different places in Ethiopia. Lobago *et al.* (2005) identified *E. brunetti* in the Kombolcha Poultry Multiplication and Research Center (KPMRC). Mersha *et al.* (2009) reported *E. tenella* in Tiyo Wereda, Arsi Administrative Zone of Oromia Regional State. *E. acervulina* and *E. maxima* were also identified in the present study. Ahmed *et al*. (2003) reported that the presence of *E.acervulina and E.maxima* species in Egypt at higher rate. Lobago *et al*.

(2005) stated that the prevalence differences were normal due to the differences in the epidemiological situation among different countries. Haug *et al*. (2008) found the incidence of *E. acervulina and E. maxima* was 100% and 27.5% in broiler chickens in Norway.

Moreover, mixed infection by different coccidian species was found in this investigation. This result agreed with the finding of Ahmed *et al*. (2012), William (1999) and Lobago *et al*. (2005), who noticed the mixed infection with different species of *Eimeria* in the chickens.

The gross pathological changes observed in the caecum of chicken positive for *Eimeria* species parasitizing the caecum were in agreement with the earlier reports of Sil *et al.* (2002). The gross lesion scoring rate (GLS) in koekoek breed was significantly higher than local and Isa brown chickens ($p < 0.05$) and this could be due to management and breed factor. Lesion score between male and female did not show significantly different. This was similar with the finding of Meskerem *et al.* (2013). Absence of statistically significant difference between male and female may be due to equal chance of exposure for the parasite infection. The severity of the macroscopic and microscopic lesions was higher in caecal portion of chickens. Similar findings were also mentioned by Talha *et al*. (2001) and Sil *et al.* (2002).

The microscopic lesions identified and characterized for identified Eimeria species in this study were almost similar with other author's findings elsewhere. Hein (1971) reported duodenal lesions similar to these findings from chicken positive for *E. acervulina.* This species showed presence of gametocyte in duodenal enterocytes with the characteristic inflammatory cells.

Eimeria tenella showed considerable numbers of schizonts or meronts in lamina propria of caecum sever hemorrhage, complete desquamation of epithelium and edema of muscular tissue. This was similar with findings reported by McDougald and Fitz-Coy (2008) and Ahmed *et al*.(2012), who described the most pathogenic *E. tenella* has the

second generation schizont, which caused excessive tissue damage, bleeding, disruption of the caecal glands and destruction of the mucosa and muscularis layer.

In chicken positive for *E. necatrix* the entire lamina propria revealed severe hemorrhages, necrosis and disintegration of glandular epithelial cells. Several schizonts were observed in the epithelial cells along with merozoites and deeply embedded in tunica musculosa and serosa, infiltrating heterophil and eosinophils. Gautama *et al*. (2010) reported similar lesions from broilers suffering from *E. necatrix.* This histopathological lesions observed in the present study were similar to the findings of Meskerem *et al*. (2013) and Talha *et al*. (2001).

The present study showed that there were cases which were scored as mild lesion at gross lesion scoring but severe microscopic lesions in microscopic lesion scoring (MLS). This result agrees with the finding of Idris *et al*. (2007) who reported that MLS detects not only oocysts but also developmental stages of the parasite and lesion. Therefore, when used alone, GLS may under estimate severity of lesion. Goodwin, (1994) reported certain chicken may lack gross intestinal lesions attributable to coccidial infection.

In support of the present observations, Johnson and Reid (1970) reported that *E.maxima* is a difficult species on which to perform GLSs because the degree of pathogenicity does not always correlate with severity of the gross intestinal lesions. In addition, gross lesions do not measure the extent of pathophysiological change in the intestinal resulting from coccidiosis (Conway *et al*., 1990). Kogut and Powell (1993) noted that the use of GLS as the primary measure of degree of pathogenicity could cause problems because large variations in GLS occur in chickens receiving equal numbers of oocysts. It is important to note that, even without gross intestinal lesions, chickens infected with coccidiosis, will perform severe microscopic lesions. Griffiths *et al*. (1987) and Conway *et al. (*1990) observed that gross lesions need not be present for chickens to suffer from significant intestinal absorptive defects.

6. CONCLUSIONS AND RECOMMENDATIONS

In present study the majority of chickens with clinical signs of coccidian were positive for *Eimeria* species. Five species of *Eimeria* were identified. Chicken positive for *E. tenella* showed the most severe lesions when compared to others and it was the predominant species isolated in the study area. Chicken with bloody coccidiosis indicated high reduction in RBC, PCV and Hb. Histopathological lesions were very suggestive of coccidiosis as the different stages of parasites together with severe tissue necrosis and inflammatory cells were observed in situ. Microscopic lesion scoring was superior to gross lesion scores for detecting coccidiosis that had been overlooked by gross lesion scoring. It can be concluded that coccidiosis had a destructive effect on chickens that is represented by high reduction in RBC value, increment in leukocyte counts, various lesions and it was found to be one of the main causes of chicken mortality in the study area.

Based on the above conclusions, the following recommendations were forwarded:

- MLS should be used together with GLS in routine coccidiosis screenings to ensure that economically significant field cases of coccidiosis are not missed and appropriate treatment can be taken to prevent further losses.
- Efforts towards the control of the disease through good management practices, good biosecurity measures and the proper use of anti-coccidial drugs should be practiced.
- Awareness should be created among farmers regarding poultry coccidiosis and its preventive and control methods.
- Further detailed experimental studies need to be undertaken to fully understand the effect of coccidiosis on the blood parameters, severity and pathogenesis of coccidiosis.

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8. ANNEXES

Annex I: Method of flotation (Permin and Hansen, 1998; Idris, 2007).

- Weigh out of feces 3 gram of faeces or the intestinal pools are suspended in 20 to 50 ml of water and mixed thoroughly
- Pour the faecal suspension through a metallic sieve in to centrifuge test tube.
- The mixture is centrifuged at 2000 r.p.m for 2 minutes and supernatant fluid is discarded.
- Pour 50 ml flotation fluid (Nacl 350g in 1000ml of tap water at Specific gravity 1.20) in to the test tube until slight convex meniscus formed at the top, which is placed in a vertical position in a test tube rack.
- Place a cover slip on the top of the test tube and leave the test tube for about 5-15 minutes, making sure no air bubbles are present. The coccidia oocyst will be floated and accumulate just beneath the cover slip.
- Lift of the cover slip vertically from the tube together with the adhering flotation fluid and Place the cover slip on a microscope slide, and examined under the microscope at 10-40 x magnification.

Annex II: Hematoxylin eosin stain procedure (Bancraft *et al.*, 1990)

1. Deparaffinize slides in 3 changes of xylene for 3 minutes each.
2. Hydrate slides in 100% alcohol and 95% alcohol, 2 changes for 3 minutes each, and rinse in distilled water until ripples disappear from slides.
3. Stain rehydrated sections in Hematoxylin solution for 20-40 minutes.
4. Wash in tap water for 1-5 minutes, until sections turn blue ("bluing").
5. Differentiate sections in 70% ethanol containing 1% HCl for 5 seconds. This removes excess dye, allowing nuclear details to emerge.
6. Rinse in tap water for 1-5 minutes until blue.
7. Stain in Eosin solution for 30 seconds - 2 minutes.
8. Wash 1-5 minutes in tap water.
9. Dehydrate in 95% alcohol and 100% alcohol, 3 changes each for 2 minutes.
10. Clear in 3 changes of xylene for 2 minutes each.
11. Mounted by Canada balsam with a cover slip

Annex III. Normal value of hematological parameters in chicken

Variable	Reference interval	Source
RBC (1×10^6 cells µl)	2.5–3.5	Wakenell (2010)
PCV (%)	35–55	Irizaary-Rovira (2004)
HB ($g.dl^{-1}$)	7–13	Wakenell (2010)
MCV (fl)	90–140	Wakenell (2010)
MCH (pg)	33–47	Wakenell (2010)
MCHC (%)	26–35	Wakenell (2010)
Total WBC ($x10^4$ µl)	1.2-3. 0	MVM(2011)
Lymphocytes (%)	34	MVM (2011)
Monocytes (%)	2.8	MVM (2011)
Eosinophils (%)	0.3	MVM (2011)
Heterophils (%)	0–1	MVM (2011)
Monocytes (%)	2.8	MVM (2011)

Annex IV: Histopathological lesion scoring of chickens infected by coccidiosis (Idris *et al.*, 2007)

Criteria	Score 1	Score 2	Score 3	Score 4
The degree of villous atrophy / villous fusion	normal villous architecture & fusion of two villi in a section	Mild Atrophic of villous but villi are still detectable & fusion of more than two villi or several fusions of two	Marked villous atrophy desquamation of villi & more than two villi were fused	Total villous atrophy with completely flat mucosa and villi are not detectable
mucosal damage Epithelial erosion	Flattening of epithelial cells in a few villus tips	Defect or parasite-erosion at tips of a few villi	Defect or parasite - erosion at tips of multiple villi	Large clusters of fused villi Severe erosions
cellular infiltration	Absent/ Mild infiltration	Moderate infiltration	Marked infiltration	Diffuse cellular infiltration
Dilation of capillaries & hemorrhages	mildly dilated & Petechial hemorrhage	Mildly dilated throughout & Slight hemorrhage	Moderately dilated & Severe hemorrhage	Severely dilated & defuse hemorrhage
Necrosis, Sub mucosal and muscular damage	Thickened, Mild destruction of layer & Few edema	reduced /disruption of sub mucosal glands & moderate edema	Reduction of sub-mucosal glands &severe edema	necrosis of glands ,severe hyperplasia of tunica muscularis & very severe edema
Presence of any coccidial stage in the four fields	one field contain parasite	two fields contain parasite	three fields contain parasite	all field contain parasite

Annex V: Blood parameters format sheet

Code	Age	Sex	Breed	Blood parameters						
				RBC	PCV	Hb	MCV	MCH	MCHC	WBC

Annex VI*: Eimeria* Oocysts and location of lesions along the intestine

Code	Parasitological findings (oocyst)			Pathological findings			*Eimeria species*
	Shape	Size	Sporulation time	Gross lesions & location	Lesion score	Histopathological Findings	