Thesis Ref No ----------------

CHARACTERIZATION OF LESIONS AND EVALUATION OF HAEMATOLOGICAL AND SERUM BIOCHEMICAL CHANGES IN SCAVENGING CHICKEN NATURALLY INFECTED BY GASTROINTESTINAL HELMINTHS IN AND AROUND BISHOFTU

MSc Thesis

BY

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Department of Pathology and Parasitology

MSc Program in Veterinary Pathology

June, 2017

Bishoftu, Ethiopia

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A thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfilment of the requirements for the degree of Master of Science in Veterinary Pathology

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As member of the Examining Board of the final MSc open defence, we certify that we have read and evaluated the thesis prepared by: Debella Taweya titled **characterization of lesions, evaluation of haematological and serum biochemical changes in scavenging chicken naturally infected by gastrointestinal helminths in and around bishoftu** and recommend that it be accepted as fulfilling the thesis requirement for the degree of master of science in

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Final approval and acceptance of the dissertation is contingent upon the submission of its corrected copy to the CGC through the concerned departmental graduate committee. I hereby certify that I have read the revised version of this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfilment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to anyother institution anywhere for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however permission must be obtained from the author.

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DEDICATION

I dedicate this thesis manuscript to my wife Kibire, My brother Tolessa and my sister Bedane. for their true family fellowship and practical influence in my life.

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LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
H and E	Hematoxylin and Eosin
MCV	Mean Corpuscular Volume
MCHC	Mean Corpuscular Hemoglobin Concentrations
MCH	Mean Corpuscular Hemoglobin
NAHDIC	National Animal Health Diagnostic Investigation Center
NMSA	National Metrological Service Agency
PCV	Packed Cell Volume
SAS	Statistical Analysis System
Spp	*Species*
TLC	Total leukocyte count
TRBC	The Total Red Blood Cell
WBC	White blood cell

ABSTRACT

A study was conducted from November 2016 - May 2017 on eighty free range chickens purchased from Bishoftu local markets, to determine the prevalence of gastro-intestinal helminths,for characterizetion of lesions of lesions in parasite positive chicken, and to evaluate change in haemathological and serum biochemical parameters. The gastrointestinal helminths were isolated and characterized. Blood sample was collected from brachial vein in vacutainer tube with anticoagulant for hematological examination and also in plain vacutainers for serum biochemical analysis. After gross lesion characterization tissues were collected into sample bottles containing buffer neutral 10% formalin for microscopic lesion characterization. From a total of 80 chicken examined by postmortem 76 (95%) were infested with one or more types of helminth parasites. Seven nematode parasites namely, *Aucaria hamulosa*, *Ascaridia galli*, *Hetrakis.dispar, Hetrakis gallinarum*, *Hetrakis isolenchae*, *Subulura brumpti, Allodapa sucturia* and 6 cestode species namely, *Raillietina tetragona*, *Raillietina cesticillus, Raillietina echinobothrida, Hymenolepis Carioca, Hymenolepis continana* and *Choenetenia infun* were identified. The diference in isolation rate was not significant between male and female (x^2=0.278, P>0.05) and among age group (x^2=0.268, P> 0.05). The frequent gross lesions include necrosis and haemorrhages in gizard,very soft feces containing much mucous exudate in the small intestine, tickened mucosal wall with petechial haemorrhage and thick white pesty mucous in the lumen. Microscopic lesions revealed degeneration and necrosis of epithelium and intestinal glands, infiltration of inflammatory cells arround the parasite attachments in intestines, excessive tissue damage, hemorrhage, and necrosis in the affected gizzard.The mean haemoglobin, total erythrocyte count, total leukocyte count and packed cell volume were 11g/dl, 2.67x10^6/ul, 3.04 x10^3/ul, 30.3% respectively in naturally infected chickens and 12g/dl, 3.5 x10^6/ul, 2.4 x10^3/ul, 36.3%, respectively in negative chicken.The mean difference was statistically significant for total erythrocyte count, total leukocyte count, and packed cell volume (P<0.05). Chickens with mixed parasites showed decreased levels of glucose and increased in total protein and aspartate aminotransferase. Means diference for the single and mixed infection was statistically significant for aspartate aminotransferase, total protein and glucose. It can be concluded that gastrointestinal helminthes are one of major problems of local backyard chicken.

Key words: *Bishoftu, Chicken, Ethiopia, Gasterointestinal helminths, Hematology, Lesion*

1. INTRODUCTION

Poultry are domesticated birds kept by man for the purpose of obtaining meat, eggs and sometimes feathers. They include birds like chicken, duck, goose and turkey. Poultry are kept in backyards or commercial production systems in most areas of the world. It is one of the most important sources of protein and farm manure, for man (Jegede *et al* ., 2015).

In Africa, 80% of chicken populations are raised under the extensive system (Guèye, 2001). This is based on a traditional undemanding mode of exploitation and it is considered as an important source of protein and income for rural communities. However, in this type of production system the chickens are with higher risk of infection by a wide variety of parasites (Fouzia *et al*., 2013).

The total chicken population in Ethiopia is estimated to be 56.5 million with native chicken representing 96.9%, hybrid chicken 0.54% and exotic breeds 2.56% (CSA, 2014). This population represents a significant portion of the rural economy, as a source of income for small holder farmers. Indigenous fowl reared under traditional extensive (rural scavenging) system and or improved traditional (semi scavenging) production system constitute one of the important component of rural economy. The traditional poultry production system is characterized by low input, low output and periodic destruction of a large portion of the flock due to gastro-intestinal parasites (Aman *et al*., 2013).

Domestic birds are highly susceptible to infection with large number of internal parasites specially helminthes one. In heavily parasitized young birds, the common manifestation are stunted growth, emaciation, weakness and death in young, while in laying hens the egg production was lowered or entirely stopped. The problems of helminthes infection in birds were discussed by many authors**,** Phiri *et al.* (2007) and Nnadi *et al.* (2010). Who recorded that delayed maturity; lowered egg production and increased susceptibility to infectious diseases were the consequences to tapeworms infestation. Beside this fact, Abrha *et al.* (2014) added that *Ascaridia galli* was the great cause of losses due to reduction in weight of chicken while tape worms constitute the most common helminthes causing severe losses, as they produce edematous and thickened wall, hemorrhagic, sloughed mucosal surface of intestinal wall.

Parasitism ranks high among factors that threaten village chicken production. Parasitism causes reduced growth, egg production, emaciation, and anaemia as well as mortality. In addition, the roles of poultry worms such as *Heterakis gallinarum* has been associated with the transmission of Histomonas meleagridis in turkeys and chicks. These parasites infect the intestines causing hemorrhage and thickening of the intestinal walls, leading to poor feed absorption and poor growth. Small roundworms are passed directly from bird to bird by ingestion of the parasite eggs or by ingestion of earthworms, insects, and other vectors carrying the parasite (Nnadi *et al.,* 2010).

Nematodes (roundworms) are the most significant in number of species and in economic impact. Of species found in commercial poultry, the common roundworm (*Ascaridia galli*) is by far the most common. Generally, nematodes have separate sexes that have morphologic differences; eg, males of *Tetrameres* spp are elongated and slender, whereas gravid females are globe-shaped. The size and shape of nematode species vary widely; ascarids are sturdy and long (up to 4.5 in. [116 mm]); capillarids are more delicate, slender, and long (2.3 in. [60 mm]); and other nematodes are much shorter (0.08–0.48 in. [2–12 mm]).

Cestodes (tapeworms) also vary in size. *Raillietina* spp may be >12 in. (30 cm), where as *Davainea proglottina* often is <0.16 in. (4 mm). The proglottids of individual tapeworms are hermaphroditic. Tapeworms have been recovered in the thousands from individual chickens.

Gastrointestinal parasitism in poultry has adverse economic effects on production parameters, more so among in backyard or farmyard flocks in comparison to confinements rearing is being adopted in modern commercial farming. Helminths eg. Round worms (Ascarids), caecal worms (Heterakis), hairworms (Capillaria) and tapeworms having invertebrate intermediate hosts or mechanical vectors play significant roles in poultry production (Solanki, 2015). In addition, processing of each specimen for the desired test with appropriate laboratory procedures and identification of the characteristics of the lesions such as its location on the intestinal tract, its appearance and severity, the nature of intestinal contents and other associated hematological and bichemical change should be studed.

Statement of the problem

In Ethiopia, poor management, nutritional deficiency and poultry diseases are the most important factor in reducing both the chicken's population and their productivity (Yami, 1995). Among poultry diseases helminthosis was considered to be the most important problem of local chickens and major causes of ill-health and loss of productivity in different parts of Ethiopia (Yimer *et al*., 2001).

In and around Bishoftu, studies related to lesions characterization, evaluation of haematological and serum biochemical changes in scavenging chicken infected by gastro-intestinal helminths was not so far conducted.

General Objective

The general objective of this study was to investigate the main gastrointestinal parasites naturally infecting the scavenging chicken and characterization of lesions that might be caused by these parasites.

Specific Objectives

The specific objectives of this study were as follows:

- To identify major species of gastro intestinal parasites harbered scavenging chicken
- To characterize gross and microscopic lesions, and assess changes in haematological and serum biochemical of infected chickens.
- To determine the association of isolated parasites lesions, haematological and serum biochemical parameters in naturally infected scavenging domestic chicken.

2. LITRATURE REVIEW

2.1. An overview on major gastrointestinal helminths of chicken

Worms are classified as Nematodes (Roundworms) and Cestodes (Tapeworms). Some parasites require an intermediary host, such as earthworms, snails, insects etc., and consequently preventing contact with these invertebrates is an obvious step in control and prevention (Leeson and summer, 2009). Nematodes are the most common and most important helminth species in poultry. More than 50 species have been described in poultry. Of these, the majority causes pathological damage to the host (Urquart *et al*., 1996).

Table : Predilection sites of major helminthes Poultry

Major Parasites	Predilection site
Nematodes	
Heterakis gallinarum	Caecum
Subulura brumpti	Caecum
Ascaridia galli	Small intestine
Capillaria caudinflata	Small intestine
Cheilospirura hamulosa	Gizzard
Capillaria annulata	Esophagus -Crop
Capillaria anatis	Caecum
Gongylonema ingluvicola	Esophagus-Crop
Cestodes	
Choanotaenia infundibulum	Small intestine
Reillietina tetragona	Small intestine, Large intestine
Raillietina cesticillus	Small intestine
Raillietina echinobothrida	Small intestine, Large intestine
Davainea proglottina	Small intestine
Trematodes	
Postharmostomum gallinum	Caecum
Brachylaema spp.	Jejunum
Prosthorhynchus spp.	Small intestine

Source: Fouzia (2013)

2.2. Characteristics of gastro-intestinal helmiths

2.2.1. Nematodes

Nematodes or roundworms are the most common internal parasites of chickens. These include *Ascaridia galli* (intestine), *Heterakis gallinarum* (ceca) and various Capillaria species (crop→intestine) found through the digestive. As a group, the nematodes are characterized by being long spindle shaped worms varying in color from off-white to creamy yellow (Leeson and summer, 2009). Nematodes are also known as round worms are a large and diverse group of worms with a various forms of habitat and life cycle. Not all these parasites will inhabit the intestine although intestinal forms are the most common. As with other helminthes infections of the intestine, heavily infected bird may show malabsorbition of nutrients, anorexia and weight lose diarrhoea and in severe cases, the worm can cause bowel obstruction and death. The nematodes of poultry are parasitic, unsegmented worms. The shape is usually cylindrical and elongated, bupt the cuticle may have circular annulations, be smooth, and have longitudinal striations or ornamentations in the form of cuticular plagues or spines. All worms have an alimentary tract. The sexes are separate. The *Ascaridia galli:* is a parasitic round worm belonging to Nematodes. *Ascaridia galli* is the most prevalent and pathogenic species, especially in domestic fowl, Gallus domesticus. It causes ascaridiasis, a disease of poultry due to heavy worm infection, particularly in chicken and turkeys. It inhabits the small intestine, and can be occasionally seen in commercial eggs. It is the largest nematode in birds. The body is semi-transparent, creamy-white and cylindrical. The anterior end is characterized by a prominent mouth, which is surrounded by three large tri-lobed lips. The edges of the lips bear teeth-like denticles (Ashour, 1994). The body is entirely covered with à thick proteinaceous structure called cuticle. The cuticle is striated transversely through out the length of the body and cuticular alae are poorly developed. Two conspicuous papillae are situated on the dorsal lip and one on each of the sub ventral lips (Lalchhandama *et al.,* 2009; Lalchhandama, 2010). These papillae are the sensory organs of the nematode. *Ascaridia galli* is diecious with distinct sexual dimorphism. Females are considerably longer and more robust, with vulva opening at the middle portion (approximately midway from anterior and posterior ends) of the body and anus at the posterior end of the body.

The tail end of females is characteristically blunt and straight. Males are relatively shorter and smaller, with a distinct pointed and curved tail. There are also ten pairs of caudal papillae towards the tail region of the body, and they are arranged linearly in well-defined groups such as precloacal (3 pairs), cloacal (1 pair), post-cloacal (1 pair) and subterminal (3 pairs) papillae. The eggs are elliptical, thick-shelled and are not embryonated at the time of deposition. They measure 73-92 x 45-57 µm (Soulsby, 1982; Ruff, 1991). Ascarids may migrate up the oviduct (via the cloaca) to become enshelled later within the egg (an aesthetic, but not a public health, problem, avoidable by careful egg-candling before the release of eggs to market). *Ascarida dissimilis* (turkey roundworm) may also migrate out of the intestine, through the portal system, and into the liver, causing hepatic granulomas (Kenneth *et al*., 2013).

Hetrakis gallinarum: is a small, white caecal worm having 3 small equal sized lips on the mouth and has 2 lateral membranes extending almost the entire length of its body. The worm has a distinct oesophagus, ending in a well-developed bulb containing a vulvar apparatus**.** The male is 7-13 mm long; having a well-developed preanal sucker and long alae with 12 pairs of papillae is positioned slightly to the middle of the body. It has a long and narrow tail with eggs that are thick-shelled, ellipsoid and unsegmented when deposited. They measure approximately 63-75 x 36-50 µm (Soulsby, 1982). *Hetraxis gallinarum* is a mild pathogen, in large numbers may cause thickening, inflammation, or nodulation in the cecal walls. Infection with *Hetrakis gallinarum* has been associated with cecal and hepatic granulomas. *Heterakis isolonche,* highly pathogenic in pheasants, may cause 50% mortality. *Hetrakis gallinarum* carries *Histomonas meleagridis*, the protozoan that causes histomoniasis.

2.2.2. Cestodes

Tapeworms belong to the phylum Platyhelminthes, class Cestoda. The tapeworms of poultry are all endoparasitic, hermaphroditic worms with a flat, long segmented body without an alimentary tract or body cavity. Poultry tapeworms may reach a length of 30 - 50 cm. They have a scolex (the head) followed by a neck. The rest of the body is called the strobila consisting of a number of proglottids (segments) developing from the neck. Each segment contains a set of reproductive organs. The number of segments differs between species. The segments furthest away from the neck mature and are detached from the body. These gravid segments contain numerous eggs which are released to the environment with the faeces (Permin and Hansen, 1998). The most commonly diagnosed Cestodes include: *Davainea proglottina* - a 4 mm Cestode located in the duodenum. *Choanotaenia infundibulum* - a 25 cm Cestode located in the distal duodenum and jejunum. *Raillietina tetragona* - a 25 cm Cestode located in the distal jejunum. *Raillietina echinobothridia* a 30 cm Cestode of the jejunum resulting in nodular granulomas and catarrhal enteritis (Simon and Emeritus, 2005).

Raillietinna cesticillus* and *Raillietinna echinobothrida: *Raillietina* is the name for a genus of tapeworms that includes helminth parasites of vertebrates, mostly of birds. Of the 37 species recorded under the genus, *Relliatina echinobothrida, Relliatina tetragona*, and *Relliatina cesticillus* are the most important species in terms of prevalence and pathogenicity among wild and domestic birds. The body of an adult Raillietina is a typical tapeworm structure, composed of a series of ribbon like body segments, gradually enlarging from the anterior end towards the posterior. It is whitish in colour, highly elongated, dorso-ventrally flat, and entirely covered with a tegument. The entire body is divisible into 3 parts, namely the head region called scolex, followed by an unsegmented neck or growth region, and then by highly segmented body proper called strobila. The scolex is a bulbous knob-like structure bearing suckers and a rostellum, which are the organs of attachment to the host. A defining structure from those of other tapeworms is a single prominent rostellum surrounded by four suckers (Lalchhandama, 2009). Further, an important diagnostic character among the different species of the genus is the number and arrangement of hooks and spines on the scolex (Dougald, 2011). Most pathogenic tapeworms are found in the small intestine; the scolex, usually buried in the mucosa, generally causes mild lesions. *Davainea proglottina* may cause weight loss. *Raillietina tetragona* causes weight loss and decreased egg production; *R echinobothrida* produces granulomas at its attachment sites (“nodular disease”) Leeson and summer (2009).

Davainea proglottina: they are also called the minute tapeworm or the small chicken tapeworm, is a parasitic worm that has chicken, turkey, guineafowl, grouse, other domestic and wild gallinaceous birds but also pigeons as final hosts. The worms are buried in the mucosa of the duodenum. The adult tape worms are small, 0.5 - 3 mm, with 4 to 9 proglottids. The eggs measure 28 - 40 µm (Permin and Hansen, 1998).

2.2.3. Trematodes

Trematodes commonly called flukes require at least one intermediate host (e.g. insects, Moluscus or other invertebrates) before they are able to complete their life cycle. Infections are mainly asymptomatic but in sever case they can cause nutritional deficiencies, weight lose and diarrhoea. They can be diagnosed by detection of eggs in the faeces, although this can be a challenge as their eggs are few in number and may not be found by routine fecal examination procedures.

2.3. Life Cycle of gastro-intestinal helmiths

2.3.1. The life cycle of gastrointestinal nematodes

Nematodes of poultry may have a direct, or an indirect life cycle. The nematodes of poultry that exhibit a direct life cycle; for example *Ascaridia galli* and *Heterakis gallinarum* do not require an intermediate host to complete their life cycle of development. The infected birds pass the helminth eggs in their droppings, contaminating the litter, feed, and water. This then possess as the main way by which the infection is transmitted (Ruff, 1991). Mechanical transmission by earthworms or cockroaches has been reported by Hall (1985) and there is no development of the larval stage inside these carriers (Leeson and summer, 2009).

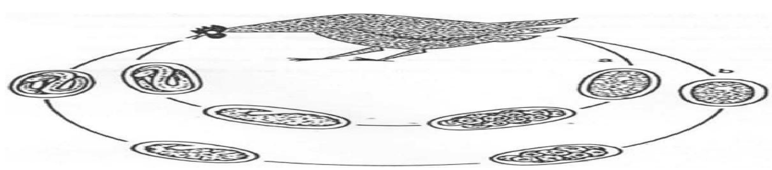

Figure 1 :- Direct Life cycle of nematodes :

Figure 2 : - Indirect life cycle of nematodes.

2.3.2. Lifecycle of Cestodes

The typical life cycle of Cestodes is indirect with one intermediate host. With few exceptions, the adult tapeworm is found in the small intestine of the final host, the segments and eggs reaching the exterior in the faeces. When the egg is ingested by the intermediate host, the gastric and intestinal secretions digest the embryophore and activate the onchosphere. Using its hooks, it tears through the mucosa to reach the blood or lymph stream or, in the case of invertebrates, the body cavity.

Once in its predilection site, the onchosphere loses its hooks and develops, depending on the species, into one of the larval stages, often known as metacestodes. When the metacestode is ingested by the final host, the scolex attaches to the mucosa the remainder of the structure is digested off, and a chain of proglottids begins to grow from the base of the scolex (Urquhart *et al .,* 1996). Mature tapeworms can be up to 25 cm in length and the continual shedding and regrowing of body segments leads to a continual drain on the nutrient reserves of the bird (Leeson and summer, 2009).

2.3.3. Lifecycle of Trematodes

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The life cycle of *Tetrameres Americana* (a) and *Subulura brumpti* (b), with embryonated eggs passed in the faeces. The eggs are ingested by the intermediate host such as cockroach, beetles, among others and within which the larvae undergoes development to the infective stage (L3). When the final host ingests the intermediate hosts, the adult worms develop in the proventriculus of the host (Ziela, 1999).

2.4. Pathogenecity and clinical signs of gastro-intestinal helmiths

Helimnthosis was considered to be an important problem of local chickens and helminthes parasites were incriminated as major causes of ill-health and loss of productivity in different parts of Ethiopia (Ashenafi and Eshetu *et al.,* 2004). In different studies the major lesions due to gastrointestinal parasites of scavenging domestic birds recorded was include enteritis characterized by hemorrhagic edematous and tick end wall of intestines that leads to disease due to serous infestation of endoparasitism (Abriha *et al.,* 2014). Endoparasites dilate the intestine, produce nodules and severe enteritis, thus impairing the absorbing power of intestine for nutrients and vitamins from the host. The resultant situation leads to loss of body weight, retarded growth, reduced egg production, weakened body resistance and even death (Salam, 2015). Parasitism is one of the major problems which inflict heavy economic losses to poultry production in the form of retarded growth, reduced weight gain, decrease egg production, diarrhoea, intestinal obstruction and poor feathers. Stress from parasites could affect the blood picture and cause anorexia (Jegede *et al .,* 2015).

2.4.1. Nematodes

Ascaridia galli infects fowl of all ages, but the greatest degree of damage is often found in young birds less than 12 weeks of age. Heavy infection is the major cause of weight depression and reduced egg production in poultry husbandry. In severe infections, intestinal blockage can occur. It results in drooping of the wings, and emaciation. It also causes loss of blood, reduced blood sugar content, increased urates, shrunken thymus glands, retarded growth and greatly increased mortality. In heavy infections, adult worms may move up the oviduct and be found in hens' eggs, and sometimes they are also found in the birds' feces (Jacobs *et al* ., 2003). Extensive *Ascarida galli* infection may reduce egg production in floor housed breeders and commercial layers. Death may occur due to intestinal obstruction in birds which are immune suppressed or are affected by an intercurrent debilitating condition (Simon and Emeritus, 2005).

The presence of *Heterakis gallinarum* also poses the danger of enhanced transmission of *Histomonas meleagridis* to both susceptible turkeys and other poultry through shedding of the eggs in the environment (Nnadi and George, 2010). Infections with Capillaria species can be highly pathogenic for birds kept in deep litter systems or in free-range systems where big numbers of infective eggs may build up in the litter or in the soil. Light infections with *Capillaria contorta* and *Capillaria annulata* produce inflammation and thickening of the crop and oesophagus. Heavy infections produce marked thickening of the oesophagus and crop wall with catarrhal and croupous inflammation. When infections occur in the small intestine or in the caeca (*Capillaria caudinflata*, *Capillaria bursata, Capillaria obsignata* or *Capillaria anatis*) the animals become emaciated, weak and anaemic. Bloody diarrhea with haemorrhagic enteritis is seen in heavy infections. *Capillaria obsignata* infections are very pathogenic in pigeons and may cause high mortality rates. Clinical signs due to *Subulura brumpti* are rarely seen, but the worm is important as a differential diagnosis to Heterakis species (Abebe *et al* ., 2016),

Oesophagus and Crop: The focus in this section is on the nematode species belonging to genus *capilaria* and the parasite *Gongylonema ingluvicola*. In heavy infection capilaria species are extremly pathogenic. There is marked thickening and inflammation with slouphing of the mucosa. The crop may become non functional causing ingluvial indigestion However under light infection, the mucosa become slightley inflammed. *Gongylonema ingluvicola* is relatively non pathogenic, it is only associated with local lesions related to the tendency to burrow under crop mucosa (Anon 1989).

Proventriculus and gizzard: The main nematodes affecting the section of GI tracts are *Dyspharynix nasuta*. *Tetrames Americana* and *Acuaria hamulosa*. These three nematodes cause do not clinical signs if the infection is light. However under heavy infection *Dyspharynix nasuta* will cause maseration of the proventricular wall *Tetramers americana* may cause emaciation and anemia. They may also lead to the inflammation of the proventricular mucosa causing complete oblitration of its lumen. Ruff (1991) and Fatihu *et al.* (1992), reported that histhopatologic sections of the proventriculus showed cystic dilation of the proventricular glands including some necrosis of the glandular epithelium. The Gizzard also seriously damaged due to *Aucaria hamulosa* tunnelling into the sub mucosa. This causes rapture mucosa with altimate formation of sac or pouch in the gizzard. Tissue degeneration of the mucosa layer there by negatively affecting the ability of the chicken to digest its feed has also been reported.

Intestines and Caecum: These sections of GI tracts are affected by *Ascarida galli, Hetrakis gallinarem, Capilaria* spp and *Allodapa sactoria*. Meanwhile, *Ascarida galli* occurs in various domasticated and wild birds in most parts of the world. Fatihu *et al* (1992) reported that *Ascarida galli* causes anaemia, decreases intestinal enzymes activity. Several cases of the adult *Ascarida galli* in the egg of domestic chicken have been reported Akinyemi *et al.* (1980). Ruff, 1991 reported that the caeca of the chickens experimentally infected with *Hetrakis gallinarem* showed marked inflammtion and some degrees of enteritis. Riddel and Gajadhar (1988) discussed the possible causal relationship between *Hetrakis gallinarem* within Caecal granulomas and development of hepatic granuloma in chicken.

2.4.2. Cestodes

More than 1400 tapeworm species have been described in domesticated poultry and wild birds. The pathogenicity of the majority of these tapeworms is unknown. A great number are harmless or have a mild pathogenicity. Few species cause severe reactions in the host (Permin and Hansen, 1998). Chronic infections due to Railllietina species are characterized by reduced growth, emaciation and weakness. Of the three species *Relliatina echinobothrida* is the most pathogenic. Nodules and hyperplastic enteritis may develop at the site of attachment. This phenomenon is named "Nodular tapeworm disease" and may occur in heavy infections. Cestodiasis results in emaciation in mature flocks, especially if severe infestation is exacerbated by malnutrition or immune suppression. *Davainea proglottina* is the most pathogenic of the poultry Cestodes, the doubly armed scolex penetrating deeply between the duodenal villi. Heavy infections may cause haemorrhagic enteritis, and light infections retarded growth and weakness *Devenia proglottina* is, despite of the small size, one of the more pathogenic species, especially in young birds and particularly if it occurs in large numbers. Clinical signs include dull plumage, slow movements, reduced weight gain, emaciation, dyspnea (difficulties in breathing), leg paralysis and death. Microscopically thickened mucosa with haemorrhages and necrosis are seen. The presence of the Cestode, *Davainea proglottina* is note worthy because of its association with haemorrhagic enteritis which could complicate anaemia of ectoparasite origin (Nnadi and George, 2010).

2.5. General Epidemiology of Nematodes and Cestodes

2.5.1. Nematodes

In Ascaridae, adult birds are symptomless carriers, and the reservoir of infection is on the ground, either as free eggs or in earthworm transport hosts (Urquhart *et al .,* 1996). Few epidemiological studies have been carried out to investigate the infection and transmission of *Ascarida galli*. It is generally accepted that the establishment of worms in the intestine is influenced by many factors such as the age of the chicken, the size of the infective dose, the age of the infective eggs, the sex of the chickens, and the diet of the host (Permin and Hansen, 1998). *Heterakis gallinarum* is widespread in most poultry flocks and is of little pathogenic significance in itself, but is of great importance in the epidemiology of Histomonas meleagridis (Urquhart *et al .,* 1996).

2.5.2. Cestodes

Poultry reared under free range conditions are likely to be infected with cestodes (tapeworms). All tapeworms of poultry have indirect life cycles with intermediate hosts such as earthworms, beetles, flies, ants or grasshoppers. The intermediate hosts are essential to perpetuate the life cycle and infections are therefore rare in indoor systems. Davainea proglottina that is found worldwide is quite common in traditional and free-ranging poultry (Permin and Hansen, 1998).

2.6. Diagnoses of gastro-intestinal helmiths

2.6.1. Nematodes

In infections with adult *Ascaridia* worms, the eggs will be found in faeces, but since it is difficult to distinguish these from Heterakis eggs, confirmation must be made by post-mortem examination of a casualty when the large white worms will be found. In the prepatent period, larvae will be found in the intestinal contents and in scrapings of the mucosa. *Hetrakis gallinarum* infection is usually only diagnosed accidentally, by the finding of eggs in faeces or the presence of worm at necropsy. *Hetrakis isolonche* infection is diagnosed at necropsy by the finding of caecal nodules containing adult worms, and if necessary, confirmed microscopically by examination of the spicules (Urquhart *et al .,* 1996). Parasitism can be diagnosed by examination of mucosal scrapings and fecal flotation, which reveal characteristic bi-operculated ova (Simon and Emeritus, 2005).

When viewed under the microscope, Nematodes have transverse grooves running across the body, but unlike the tapeworms they do not physically segment and so only the complete worms are found in the intestine or feces. Female worms produce eggs which are deposited in the feces (Leeson and Summer, 2009)

2.6.2. Cestodes

Numerous Cestode species may occur in the intestinal tract and can be diagnosed at post-mortem or by examination of feaces (Simon and Emeritus, 2005).

2.6.3. Trematodes

They can be diagnosed by detection of eggs in the faeces, although this can be a challenge as their eggs are few in number and may not be found by routine fecal examination procedures (Simon and Emeritus, 2005).

3. MATERIALS AND METHODS

3.1. Description of the study area

The study site is situated in Bishoftu town which is located 45 km in east of Addis Ababa at 9°N latitude and 4°E longitudes, at altitude of 1850 m above sea level in the central Oromia region. The area has an annual rainfall of 86.6 mm, of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures are 26 and 14°C, respectively, with mean relative humidity level of 61.3% (NMSA (2011)

3.2. Study design

A cross sectional study design and Simple random (lottery based) sampling of candidates from chicken in the market conducted. Sex and different age groups were included proporsionally.

3.3. Study animals and management

Study animals were apparently healthy local chickens (41 males and 39 female), randomly selected and purchased from local markets. The age of chicken were categorized as 7-15 Wks (34) or growers and >16 Wks (46Adults). All the chickens were transported alive in cages to the Department of Veterinary pathology and Parasitology, Addis Ababa University, college of veterinary medicine and agriculture, Bishoftu, Ethiopia.

3.4. Sample size determination

Sample size was calculated according to Thrusfield from the study of Hyredine *et al*. (2012) the 89.5% prevalence was taken as expected prevalence. The 95% level of confidence, and 5% precision was used. The sample size was as per the following formula. Accordingly, 150 chicken were needed but bacause of financial limitation only 80 chickens (both sexes) and (adult and young) were parchased from markets in Bishoftu included.

Thrusfield Formula $n = \frac{z^2 (Pexp) (Pexp)}{d^2}$

Where : n = number of study animals

Pexp = Expected prevalence

Z = 1.96 for 95% confidence interval

d = (5%) level of precission

3.5. Sample collection and sample processing

3.5.1. Blood Collection

One ml of blood was collected into vacutainer tube wih Ethylenediaminetetraacetic acid (EDTA) from wing veins for hematological parameters assement, and 6ml of blood sample was collected in plain vacutainers for biochemical analysis, as discribed by Ross *et al.* (1976) and Kavitha (2016).

3.5.2. Tissue Sample Collection :

Necropsy examination was carried out on chicken and representative tissue sample of Gizzard, duodenum, ileum and cecum were collected into sample bottle with 10% neutral formaline for histopathological examination (Bancroft and Gamble, 2002).

3.5.3.. Examination of GI tracts for helminths

Each gastrointestinal tract was spread on disecting board and separated in diferent sections. After dissection, alimentary canal was opened, followed by systematic necropsy examination which included the esophagus to the gizzard, the small intestine (duodenum, jejunum and ileum), the caeca, and the ileocaeca-colic junction to the cloaca, the lumen of each section was opened longitudinally ond the contents was scrapped into petridish containing 0.9% physiological saline as described by Fatihu (1991). The contents of each section was observed under light microscope for helminths. Helminths from each sections were isolated and preserved in lebelled vials containing formaline. The helminths wer examined and identified as discribed by Soluby (1982) and Yacob *et al*. (2009).

3.5.4. Hematological analysis

The Hb concentration is evaluated by matching acid hematin solution against a standard colored solution found in Sahl's hemoglobin meter according to the methods described by Dein (1984). Packed cell volume (PCV) was done by capillary hematocrit method. Total Red blood cell (RBC) count was determined by the haemocytometer method. Total leukocytes and leukocyte differential counts were similarly evaluated according to the method discribed by Dein (1984).

The Total red blood cell count (TRBC)

The total red blood cell count (TRBC) was performed in 1:200 dillusion of blood in Haym's solution. Blood was taken up to 0.5 level in a RBC diluting pippet. Haym's solution was suck up to 101 mark shifting the blood from the stem to the bulb of the RBC pippet. Mechanically pippete was shaken throungly by holding the pippete in between the index finger and thumb. On a clean neubar haemocytometer counting chamber, a drop of diluted blood was placed. The cells were stablized for 1-2 minutesand total red blood cells in each mm^3 area were counted under low maginification (10x); and the total red blood cells were determined by mannual method using hemocytometer according to Dein (1984).

Total leucocyte count (TLC)

Total leucocyte count (TLC) was also determined by taking the fresh blood up to 0.5 level in a WBC diluting pippet. Glacial accetic acid was suck up to 11 mark shifting the blood from the stem to the bulb of the WBC pippet. Mechanically pippete was ratated gently by holding the pippete in between the index finger and thumb. On a clean neubar counting chamber, a drop of diluted blood was placed. The cells were stablized for 1-2 minutesand total white blood cells in each mm area were counted under low maginification (10x); and the total white blood cells were determined by mannual method using haemocytometer according to the procedures set by Dein (1984).

Hemoglobin dtermination

The Hb concentration is evaluated by matching acid hematin solution against a standard colored solution found in Sahl's hemoglobin meter according to the methods described by Dein (1984). The Sahli method is based on converting haemoglobin to acid haematin (brown colour) and then visually matching its colour against a solid glass standard. Diluted hydrochloric acid is mixed into a graduated cylinder with 20ul of blood sample and distilled water is added until the colour of the diluted blood sample matches the glass standard. The dilution was determined by the Haemoglobin level of the blood sample as described by (Philippe, 2009).

Packed Cell Volume (PCV) and Blood indices

Packed Cell Volume (PCV) was measured using microhaematocrit reader from microhaematocrit (75x16 mm) capillary tubes filled with blood and centrifuged at 12,000 rpm for 5 min. The Mean corpuscular volume (MCV), and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated from total RBC count, PCV and Hb as described by Ibrahim (2013).

Differential leukocyte counts (DLC)

Blood smear were made and air-dried after preparation. Slides were fixed in methanol for 5 minutes and then stained with working Giemsa solution for 35 minutes, washed with tap water, blotted and examined under the microscope for deffirencial leukocyte counts using 100× microscopy. Each cell were counted untill 100 white cells were counted and the percentage of each WBCs was determined (Nemi, 1986).

3.5.5. Biochemical analysis

Collection of blood was carried from brachial vein and 6ml of blood, was obtained and serum was separated after centrifugation at 3,000 rpm for 5 min and stored at -20 $^0{}_C$until used.The serum total protein was measured using Biuret method. Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Alanine aminotransferase (ALT) were determined using a photoelectric colorimeter (Gallenkamp and Sons Ltd; England). Serum urea and serum creatinine were evaluated similarly using photoelectric colorimeter according to the manufacturer's instruction andas described by (Josue (2015).

3.5.6. Tissue processing and examination

After recording the gross changes pieces of intestines from the infected birds were collected and fixed in 10% buffered formaline.Histopathology was done at the National Animal Health, Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia. The fixed tissue sample was trimmed and processed in an automatic tissue processor in different chambers containing different alcohol concentrations (70, 80, 95 and 100%, 100%, 100%, 100%), cleared in xylene and embedded in paraffin for preparation into fine blocks. Blocks was sectioned at 5 µm, dewaxed, rehydrated and stained using haematoxyline and eosin (H & E) stain *(*Bancroft and Gamble, 2002*)*. The slides were mounted with Canada balsam and allowed to dry before examination under a light microscope.

3.6. Data Analysis

The data collected was entered in to Microsoft Excel spread sheets and analyzed using STATA version13 statistical software's. Descriptive statistics (frequency and percentages) were (Mohammed, 2014). The association of age and sex with helminths infection in the intestinal tract was assessed Chisquare (X^2) test).The statistical analysis system (SAS, 2000) was used to determine the the mean, range and standard deviation of hematological data. The level of the mean values of the infected and none infected were determined using t-test and a $P<0.05$ (Steel and Torrie 1982).

4. RESULT

4.1. Overall prevalence of parasitosis

Atotal of 80 chickens weres studied, of whom 76 were found to be infected with intestinal parasites, a prevalence of 95%. The identified parasites included 7 species of nematodes (*Aucaria hamulosa*, *Ascaridia galli*, *Hetrakis dispar, Hetrakis gallinarum*, *Hetrakis isolenchae*, *subulura brumpti* and *Allodapa sucturia*) and 6 cestode species (*Raillietina tetragona*, *Raillietina cesticillus, Raillietina echinobothrida, Hymenolepis Carioca, hymnolepis continana* and *Choenetenia infun*). From a total of 80 chicken examined by postmortem 76 (95%) were infested with one or more types of adult helminth parasites. The frequencies and percentages of individual parasites among the infected chickens are shown in (Figure 1).

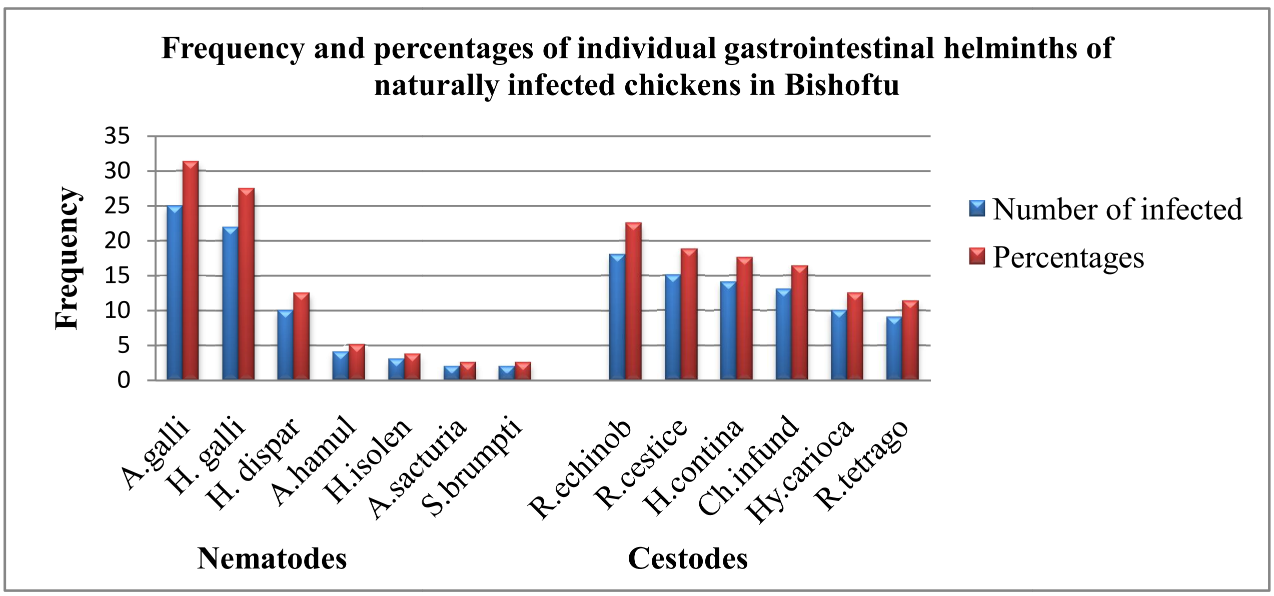

Figure 3 :-Species and frequency of gastrointestinal helminths identified from naturally infected free ranging chickens

4.2. Parasitic infections in relation to chicken sex and age

Sixty two percent females (24/39) females and 42% (17/36) of males were positive for Nematode infection. Similarly, Sixty two percent females (24/39) and 65.8% of male (27/41) chickens were positive for Cestode infection. The details of chicken sex and age wise prevalences and types of parasites identified were presented in table 2 &3 below.

Table 2: sex and age wise prevalence of individual nematode species

	No of examined	No (%) of Possitive						
Variable catagories		***Ascarida galli***	***Hetrakis gallinar.***	***Hetrakis dispar***	***Hetrakis isolenchae***	***Aucaria hamulosa***	***Subulura brumpti***	***Allodapa Sucturia***
Sex								
Female	39	13 (33.3%)	13 (33.3%)	2 (5.1%)	0 (0.0%)	1 (2.6%)	1 (2.6%)	1 (2.6%)
Male	41	12 (29.3%)	9 (22%)	8 (19.5%)	3 (7.3%)	3 (7.3)	1 (2.4%)	1 (2.4%)
Total	80	25 (31,2%)	22 (27.5%)	10 (12.5%)	3 (3.7%)	4 (5%)	2 (2.5%)	2 (2.5%)
x^2 (*p-value*)		0.52 (0.88)	0.25 (0.31)	0.05(0.088)	0.85 (0.24)	0.33 (0.62)	0.97(1.00)	0.97(1.0)
Age								
Grower	34	10 (29.4%)	9 (26.5%)	6 (17.6%)	2 (5.9%)	1 (2.9%)	0 (0.0%)	0 (0.0%)
Adult	46	15 (32.5%)	13 (28.3%)	4 (8.7%)	1 (2.2)	3 (6.5%)	2 (4.3)	2 (4.3%)
Total	80	25 (31.6%)	22 (27.5%)	10 (12.5%)	3 (3.75%)	3 (3.75%)	2 (4.3%)	2 (4.3%)
x^2 (*P-value)*		0.76 (0.81)	0.86 (1.0)	0.23 (0.31)	0.39 (0.57)	0.47 (0.63)	0.23 (0.5)	0.22(0.5)

There is no associasion in prevalence between individual nematode species and the risk factors (Sex and Age) of the parasite positive chickens (p>0.05).

Table 3: The prevalence of individual cestode species in relation to host (sex and age) in Bishoftu.

	No of examined	**No (%) of possitive**					
Variable catagories		***Relliatina. echinob***	***Relliatina Cesticillus***	***Relliatina tetragonal***	***Choenotenia Infundibulum***	***Hymnolepis Carioca***	***Hymnolepis Continana***
Sex							
Female	39	6 (15.4%)	6 (15.4%)	6 (15.4%)	6 (15.4%)	2 (5.1%)	7 (17.9%)
Male	40	12 (29.3%)	9 (22%)	3 (7.3%)	7 (17.1%)	8 (19.5%)	7 (17.1%)
Total	80	18 (21.3%)	15(18.8%)	9 (11.3%)	13 (16.3%)	10 (12.5%)	14 (17.5%)
x^2 (*p-value*)		0.12 (0.18)	0.45 (0.57)	0.25 (0.3)	0.84 (1.0)	0.52 (0.088)	0.92 (1.0)
Age							
Grower	34	8 (23.5%)	11(32.4%)	3 (8.8%)	5 (14.7%0	4 (11.8%)	5 (14.7%)
Adult	46	10(21.7%)	4 (26.7%)	6 (13%)	8 (17.4%)	6 (13%)	9 (19.6%)
Total	80	18 (21.3%)	15 (18.8%)	9 (11.3%)	13 (16.3%)	10 (12.5%)	14 (17.5%)
x^2 (*p-value*)		0.85 (1)	0.01 (0.06)	0.56 (0.73)	0.75 (1.0)	0.86 (1.0)	0.57 (0.76)

There was no significant difference observed (P >0.05) on the postmortem prevalence of cestode parasites between all risk factors that was considered (Table 3).

Table 4: Frequency and percentages of Mixed infections in relation to sex and age with Mixed infection (N=80)

Variable	No. of examined	Mixed infection (%)	x^2 (*p-value*)
Sex			
Female	39	21(53.8%)	0.273 (0.362)
Male	41	27 (65.9%)	
Age			
Grower	34	18 (52.9%)	0.268 (0.356)
Adult	46	30 (65.2)	

Helminth infection was more prevalent in males (65.9) than females (53.8%), and in adults (65.2%) followed by growers (52.9%). There was however no statistically significance difference (P>0.05) in the prevalence of mixed infection between sexes and age groups of the chicken (Table 4).

Table 5: Frequency and percentages of lesions of local scavenging chickens in reletion to sex and age of host.

Variables	No Examined	Number with lesion	Percentages (%)
Age			
7-15 Wks (Growers)	34	7	20.6
>16 Wks (Adults)	46	18	39
Sex			
Male	41	13	31.7
Female	39	12	30

Based on the above result, the gastrointestinal lesions were observed in 25/76 (32%) of examined parasite possitive chicken of which 18/46 (39%) were from adult and 7/34 (20.6%) from grower and Between sexes, male chickens seems to have hiegher frequency of lessions 13/41 (31.7%) than female 30% (12/39) (Table 5).

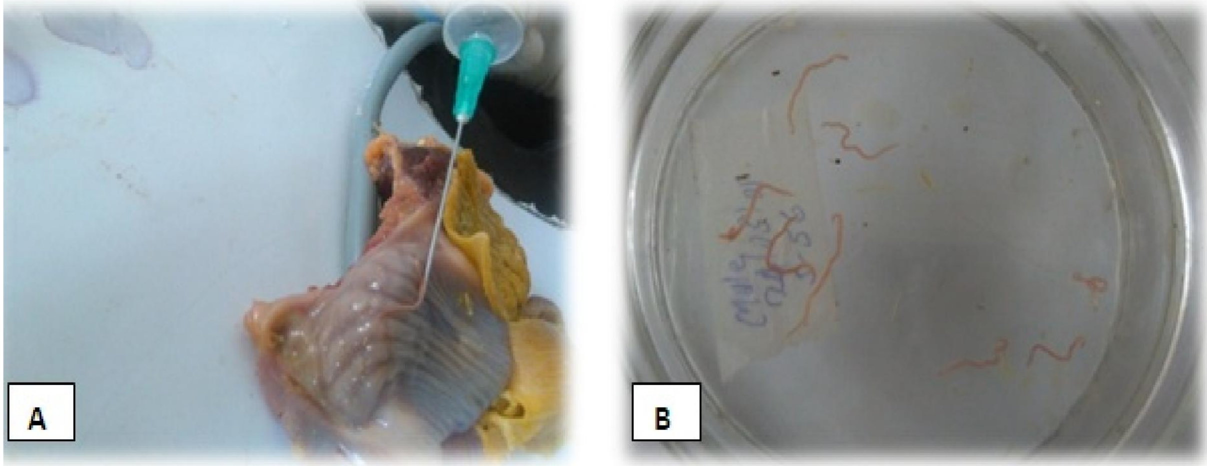

Figure 4 : - Adult *Aucaria hamulosa* worm, burrowed in the mucosa of gizard (**A**), Adult *Aucaria hamulosa* (red colored worms) isolated from the Mucosa of gizzard (**B**).

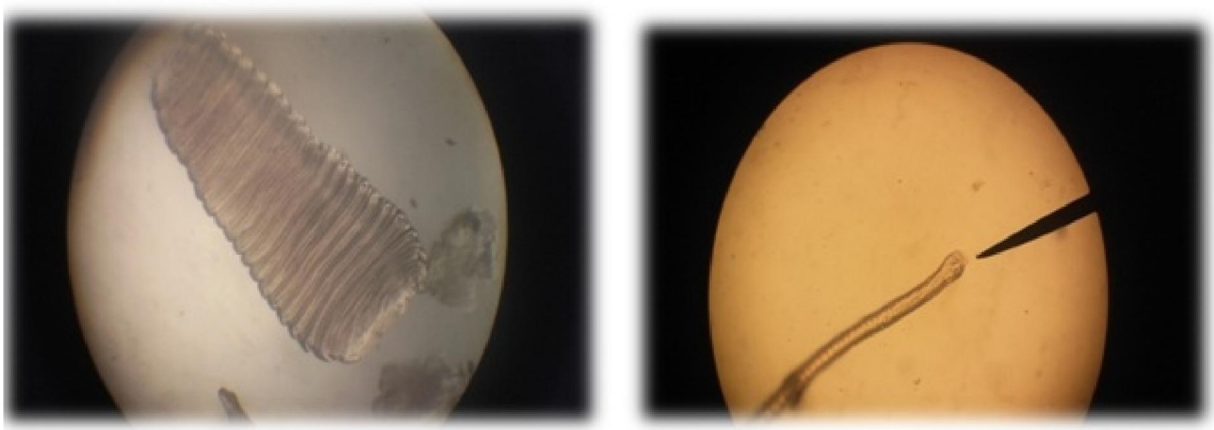

Figure 5:- Immature (Left) and Mature (Right) *Rellietina cesticillus* Collected from the Small intestine of local scavenging chicken (10x).

4.3. Lesions due to gastrointestinal helminths

4.3.1. Macroscopic lesions

Necropsy examination revealed various gross lesions in gizzard,deodenum, jejjinum, ilium, caecum, and large intestine (the details of lesion types and frquency were presented in table 6). The major gross lesions recorded include enteritis characterized by haemorrhagic, oedematous and thickened wall. The major gross lesion of gizzard infested *Aucaria hamulosa* is petechial haemorrhage, thickening of the mucosa and haemorrhages into the lumen in some cases (fig. 9).

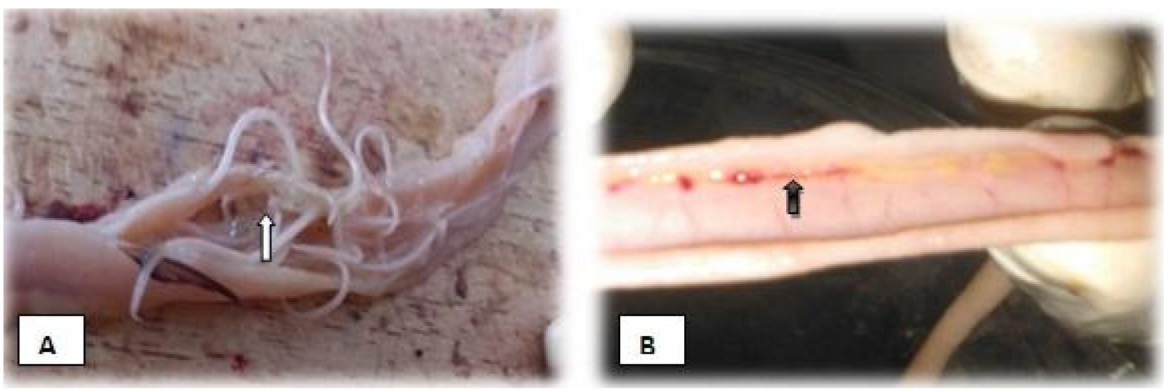

Figure 6: *Ascaris galli*almost blocking the intestine **(A)** and associated enteritis characterized by haemorrhage and thickened intestine (**B**).

Figure 7: *Relliatina .spp* in the inintestine. Note edematous fluid and thickened mucosal surface**.**

Figure 8 : Ilium containing a large number of *Relliatina spp* with hemorrhagic and slouphed mucosal layer.

Table 6: Frequence and percentages of gross lesions in different intestinal segments of infected chickens by gastrointestinal helminths.

Intestine Segments	Gross findings	Name of Parasites	Frequency	
			Number of Lesions	**Percentages (%)**
Gizzard	Thickening, Necrotized and hemorrhagic in the mucosa	*Aucaria. hamulosa*is	4	5
Deodenum	Soft to liquid consistency of the intestinal mucosa and nodules in the site of attachement	*Relliatina echinobotrida*	5	6.6
Jejjunum	Greenish or yellowish faeces with a very soft to liquid consistency and containing much mucous exudate	*Raillietina cesticillu*s	6	7.9
Ilium	Hemorrhagic and sloughed mucosal surface	*Relliatina echinobotrida*	5	6.6
Cecum	Hemorrhagic and thickened mucosal surface	*Hetrakis gallinarum*	4	5

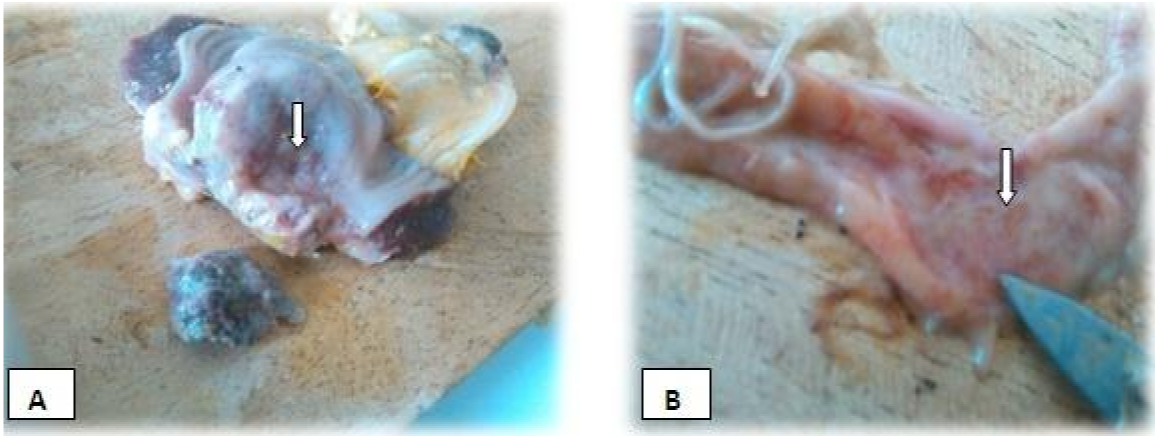

Figure 9: Petechial haemorrhages and thickening of mucosa of gizzard affected by *Aucaria hamulosa* (a) and *Ascarida hamulosa i*nduced enteritis characterized by haemorrhagic, edematous and thickened wall (b).

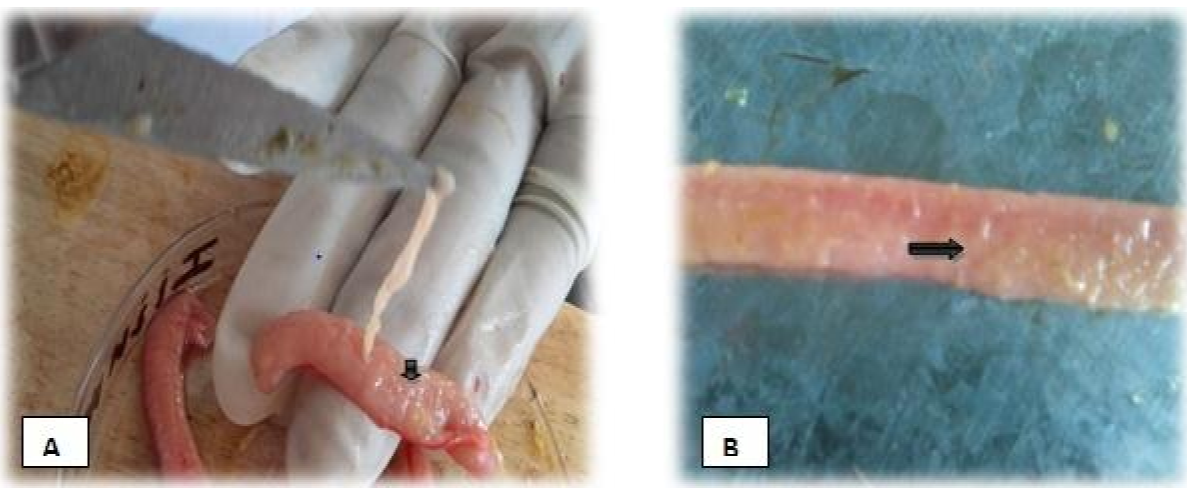

Figure: 10: - *Rellitina Echinobotrida* (**A**) with associated edematous mucosa of the duodenum. Nodules in the site of attachement of *Rellitina Echinobotrida* **(B).**

4.3.2. Histopathological Examination

Total number of 60 tissue samples were collected for evaluation of microscopic lesions. Lesions of the intestines were characterized by varying degrees of degenerative changes to sloughing of mucosa in heavy and multiple infestations. The microscopic lesions in gizzards of chickens infected with *A. hamulosa*, were seen with sever inflammatory nodules around the parasite, mononuclear cell infiltration and sections of the parasites surrounded by inflammatory cellsmainly of lymphocytes and plasma cells.

In severe cases of gizzard infested with *Aucaria hamulosa* sevre necrosis and removal of cells around the parasite, celluar debris and uniformly esosinophilic edema (figure 11).

Microscopically, unlike gizard which is characterized by necrosis and loss of cells around parasites, in the instines were heavy infiltration of inflammatory cells predominant heterophils and eosinophils..In some cases especially in the areas of mechanical damage by scolices there were infiltration by mononuclear cells, chiefly lymphocytes (Fig. 15).

Figure 11:- A corss section (black arrow) and longitudinal section (Red arrow) of *A. hamulosa* in the gizzard. Note that cells around the cestode were totally necrotized (White arrow).

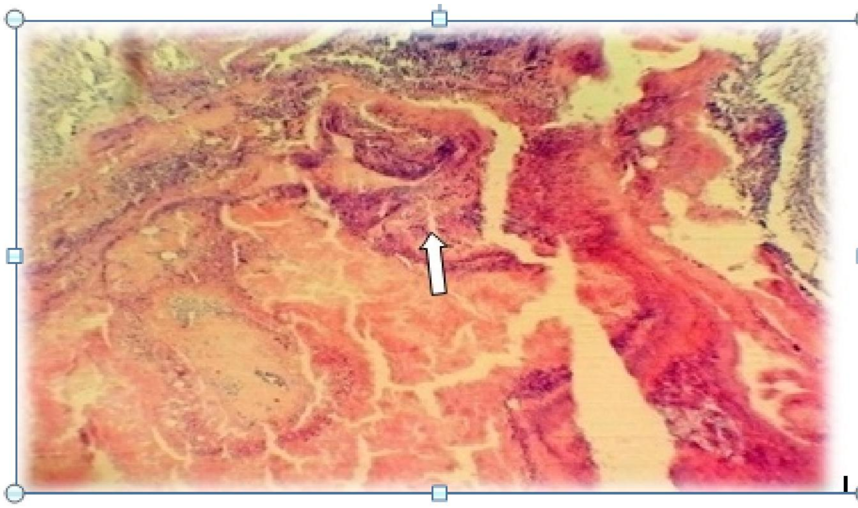

Figure 12: Multifocal granulomatous inflammation with cellar debris at the center (arrows) and eosinophilc staing edema

The inflammatory reaction was characterized by predominant heterophils, especially in the areas of mechanical damage by scolices Sparse infiltration of mononuclear cells, chiefly lymphocytes, and eosinophils was observed through out the mucosa (Fig 14**).** In cases with parasitic associations, high inestation of longitudinal and cross sections of other cestodes were observed in the disintegrated mucosa.

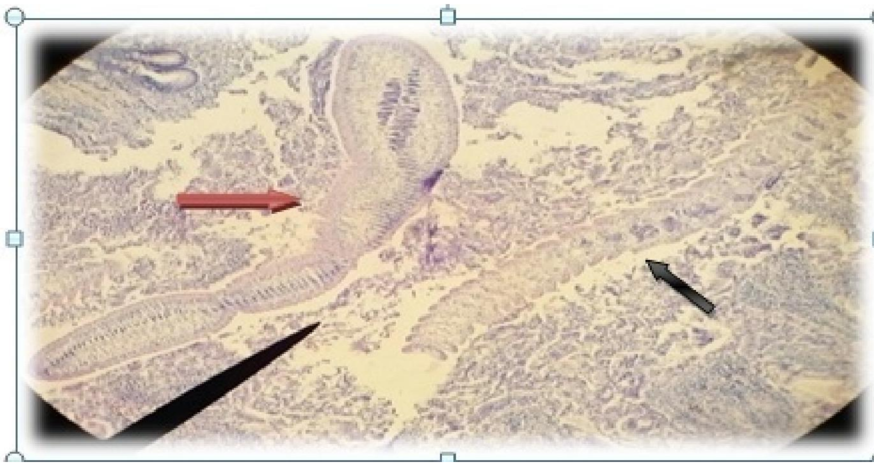

Figure 13: Picture of jejunum harboring immature (red arrow) and mature *R.cesiticellus* (black arrow), embedded deep into the intestinal mucosa causing sevre necrosis of cells leaving cyctic structure around the cestode.

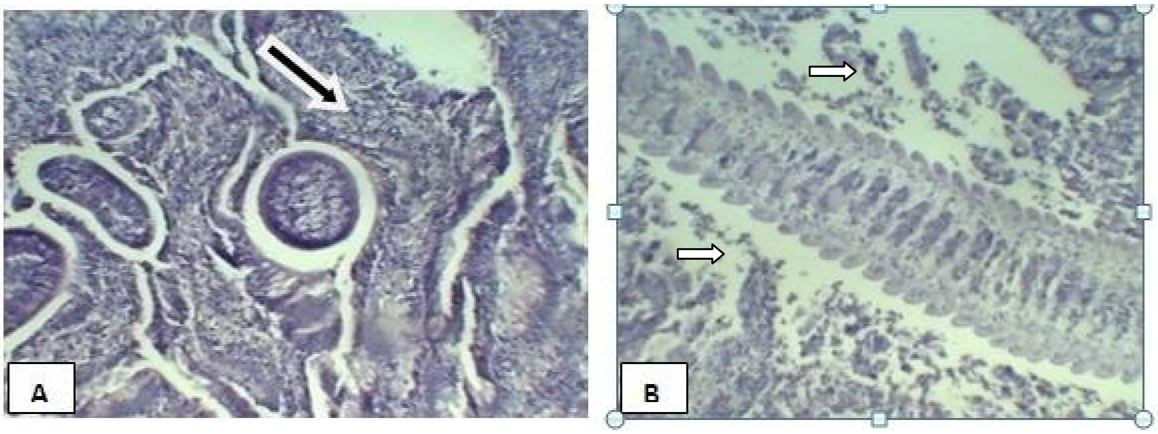

Figure 14: (a) Heavy inflammatory cells aroud the parasite. (b) Necrosis of mucosa of duodenum around the parasite and ilium epithelial necrosis.

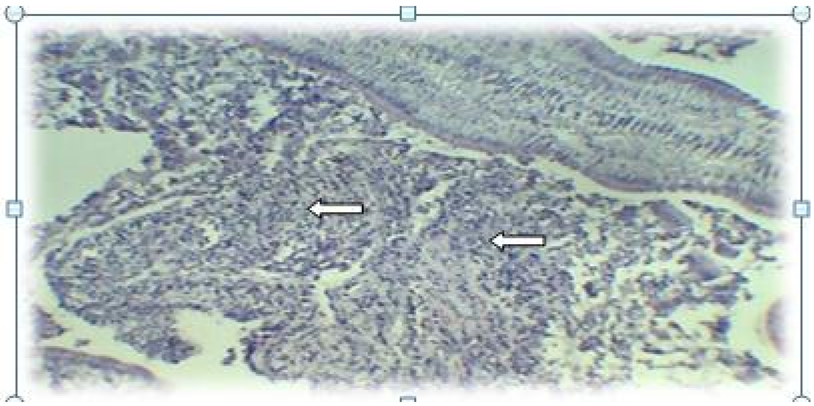

Figure 15: - Heavy inflammatory cells around the cestode. Necrosis of intestinal cells and infiltration of eosinophils into the area.

4.4. Changes in haematological pattern in infected and non infected chicken

Chickens negative for intestinal parasites had a mean PCV and total red cell value of 36.3% and 3.57 $x10^{6/}$ul which was significantly higher than the value of 30.8% and 2.67$x10^{6/}$ul parasite positive chicken. The diffrence in the mean value was statistically significant ($P<0.05$).The mean value of total leukocytes count was 2.47 $x10^{3/}$ul in non infected chickens and 3.04 $x10^{3/}$ul) in chickens with parasitic infections. The difference in mean was statistically significant ($P < 0.05$). Similarly the mean difference in MCV, hetrophil, lymphocytes and eosinophils counts were statistically significant ($P<0.05$) beteween parasite negative and parasite positive chicken (Table 7).

Table 7: The mean haematological parameter of positive and Parasite negative chickens.

Parameters	**Parsite negative chicken (N=4)**	**Parasite Possitive chicken (N=76)**	***p-value***
	Mean ± SE	**Mean ±SE**	
PCV (%)	36.3±4.35	30.80±0.71	0.04
RBC X $10^{6/ul}$	3.57±0.04	2.67±0.06	0.002
WBC X 10^3/ml	2.47 ± 0.20	3.04±0.08	0.04
MCV (fl)	87.7±19.3	116.6±19.75	0.005
MCH (pg)	43.8 ± 5.45	38.68±.84	0.08
MCHC (%)	33.47±4.60	36.15±5.45	0.83
Hetrophils	7.5±3.32	13.76±6.8	0.03
Lymphocyte	31.5±4.65	41.5±5.59	0.004
Monnocyte	1±0.81	2.69±1.69	0.97
Eusinophil	12±7.16	18.2±7.46	0.04
Basophil	0.0±0.0	0.19±0.04	0.83

RBC= Red blood cells, PCV = Packed cell volume, HG = hemoglobin, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration

Means difference of hematological value between the Parasite negative and parasite positive of chickens was significantly different ($p < 0.05$), for PCV, RBC, WBC, MCV, Hetrophils, Lymphocytes and Eusinophils (Table 7).

Table 8: Mean of hematological parameters by sex and age of local chickens

Parameters	Sex			Age		
	Female	**Male**	**p-value**	**Grower**	**Adult**	**p-value**
PCV (%)	31.4±0.97	30.7±0.98	0.30	30.8±0.78	31.2±1.06	0.39
Hb (g / dl)	11.5±0.35	10.8±0.35	0.10	11±0.37	110.25	0.27
RBC (× 106 / µl)	2.5±0.09	2.8±0.09	0.94	2.59±0.09	2.80±0.09	0.9
WBC (× 10/ µl)	3.00.09	2.97±0.11	0.27	2.96±0.10	3.0±0.10	0.05
MCV (fl)	111.3±3.14	118.8±3.29	0.05	121.2±3.55	110.7±	0.01
MCHC (g)	36.06±0.91	35.96±0.81	0.4	37.2±0.88	35.13±0.80	0.04
Heterophils (%)	12.1±0.95	14.7±1.14	0.09	13.6±1.24	13.2±0.96	0.40
Lymphocytes (%)	40.2±1.01	41.7±0.86	0.87	42.5±0.90	39.8±0.91	0.01
Monocytes (%)	2.15±0.22	3.04±0.28	0.99	2.76±0.29	2.5±0.24	0.24

RBC= Red blood cells, PCV = Packed cell volume, Hb = hemoglobin, MCV = Mean corpuscular volume, MCHC = Mean corpuscular hemoglobin concentration.

Means difference hematological value between the sex of chickens was not significantly different ($p > 0.05$), but the mean of MCV, MCHC and Lymphocytes were higher and statistically different between growers and adult ($p<0.05$) chickens (Table 8).

4.4.1. Serum biochemical parameters

Table 9:- The means of serum biochemical changes of infected and non- infected group

Parameters	**Negative**	**Possitive**	***p-value***
Alanine aminotransferase (ALT/ul	30.8±0.70	36.25±2.17	0.04
Aspartate aminotransferase (AST/ul)	420.9±54.65	341.3±19.36	0.09
Alkaline phosphatase (ALP/ul)	198.8±58.27	552±93.65	0.01
Total protein (g/dl)	7.75±3.32	19.8±1.74	0.01
Glucose (g/dl)	245.2±22.3	226.8±8.5	0.01

ALT =Alanine aminotransferase, AST = Aspartate aminotransferase, ALP =Alkaline phosphatase

Means difference in most biochemical parameters for the two group was significantly different ($p < 0.05$), namely for, ALT, ALP, total protein and glucose.The mean of ALT, ALP, and total protein were higher in infected group, while the mean value of glucose was decreased in infected group (Table 9).

Table 10 :- The means of serum biochemical parameters in (single and mixed) type of infection

Type of infections	Single infection (N=17)	Mixed infection (N=19)	(p-value)
	Mean ± SE	**Mean ± SE**	
ALT	19.54 ± 4.98	31.93 ±13.02	0.20
ALP	460.52 ± 125.63	634.07±137.73	0.18
AST	309.72 ±26.48	376.55 ±26.55	0.04
Glucose	236.29 ± 10.03	227.43 ±13.67	0.017
Total Protein	3.42± 1.74	4.70±0.83	0.04

ALT=Alanine aminotransferase, AST = Aspartate aminotransferase, ALP =Alkaline phosphatase

Chickens positive for multiple gastrointestinal parasites showed decreased levels of glucose and increased in total proteinand aspartate aminotransferase (AST) concentration in serum compared to those single parasite infection. Means diference for the single and mixed infection was diferent for AST, Total protein and glucose ($p<0.05$)

5. DISCUSSION

High prevalence of gastrointestinal helminths (95%) in this study was slightly higher than 89.9% of Heyradin *et al*. (2012) from Eastern Shewa Zone. However the result wascomparable with 97% of Senyonga (1982) from ganda, Fatihu *et al*. (1991) 95.7% from Nigeria and 95.2% of Phiri *et al*. (2007) from Zambia,.These studies indicated also multiple infections with helminth parasites, which is in agreement with our observations.The high rate of *Ascaridia galli* infection in this study may be due to environmental conditions such as moisture which supports larval development and facilitate transmission (Audu *et al*., 2004; Ogbaje *et al*., 2012) or it might also be dueto poor biosecurity, poor hygiene and management.

From 39 female and 41 male chickens examined by postmortem, 24/39(62%) and 27(65%) were found positive for adult cestode parasites respectively. This study was in agrement with the reports from Hawassa and Shashemene by Beruktayet *et al.* (2016), who reported 12(63.1%) and 14(66.7%) of prevalence of cestode species in female and male respectively.

The lower prevalence of helminths in female chickens recorded in this study agrees with the reports of Adang *et al.* (2014) but contradicts that of Farjana *et al*. (2004) and Yousuf *et al*. (2009). This could be due to the fact that female chickens reduce their feeding habbits during their incubation periods and most farmers take special care of incubating chickens by giving them feeding like grains and foods reminants and water to compansate for the time spent in incubation Yoriyo *et al*. (2008), and this reduces ther chance of picking infections. The male chickens being free increasing their food and mate, thus increasing their chance of picking infections and are there fore more exposed to helminths infections than females.

In this study no significant diference was observed in parasitic infection (prevalence of each species due to the variation in hosts sex and age). As was observed in previous studies done by Magwisha (2002), Ashenafi and Eshetu (2004), the result of the present study showed that it seems no natural affinity of helminth species to either sex or age of the host.

The gastrointestinal lesions were observed in 25/76 (32%) of examined parasite possitive chicken of which 18/46 (39%) were from adult and 7/34 (20.6%) from grower and Between sexes, male chickens seems to have rate of which 13/41 (31.7%) higher than female chickens 30% (12/39). These findings were in agreement with the findings of (Beruktayet *et al*., 2016) in which adult birds were compared to young birds. The common microscopic lesions of positive chickens were degeneration of the epithelium, in sever cases necrosis of the epithelium and intestinal glands were degenerated at the site of infection. The predominant inflammatory cells were heterophils in some cases and lymphocytes and proliferation of connective tissues suggestive in more chronic cases. Also hetrophil, lymphocytes and eosinophils were seen in the sub mucosa. Infiltration of mononuclear cells and eosinophils in the intestinal tissue was also recorded in the later stage of infection (Gray, 1975). Besides these various other reports also indicate that the tapeworm infection causes leucocytosis, eosinophilia and heterophilia (Matta, 1980).

Acuaria hamulosa were found embedded in nodules in the gizzards of the infected chickens. The microscopic lesions was higher in adults among the age groups than growers.. The microscopic lesions in gizzards of chickens infected with *Aucaria hamulosa* were necrosis and lysis of the cells around the parasites. In some there were mononuclear cell infiltration and sections of the parasites surrounded by lymphocytes. Other changes were epithelial desquamation, haemorrhages and infiltration in the affected tissue by plasma cells (Sheikh *et al* ., (2010).

Attachment of the parasite caused traumatic lesions which might favor secondary bacterial infection and hence more severe cellular reaction in the area. However local effects and species specific studies are needed. The higher prevalence of the parasites and observed pathology directly reflects their economic importance and warrants conscious intervention for its control in backyard poultry.

The mean PCV and RBC counts were significantly reduced while WBC, MCV, hetrophil, lymphocyte and eusinohil in positive chicken where increased with asignificant difference ($p<0.05$) when compare with the negative chickens. The high infection of intestinal parasites are strongly associated with the development of anaemia as they cause malabsorption, nutritional deficiencies and gastrointestinal blood loss (Ejezie *et al* ., 1993). Similar findings were reported from chicken infected by cestode parasites that causes reduction in PCV, RBC count haemoglobin concentration (Aade *et al.,* 2012). This likely would be result of the combined effect specially deficiency of

Vitamin B12, that may result in formation of large but few RBC, malabsorption and gastrointestinal blood loss due to infection with gastrointestinal intestinal parasites (Aade *et al.,* 2012).

The mean increase of lymphocytes, monocytes, eosinophils and heterophils were in positive chicken than in negative chicken. Similar finding was reported by Ricklefs and Sheldon (2007), who found the high counts of lymphocytes, heterophils and eosinophils in parasitic (malaria and haemosporidin) infected chicken. The increase in the lymphocyte count may be attributed to the effect of the inflammation of the caeca and intestine. Chronic antigenic stimulation may result in a greatly expanded circulating lymphocyte pool because the primary functions of the lymphocytes are immunological response, humoral antibody formation and cell mediated immunity (Irizaary-Rovira, 2004). Wakenell (2010) noted that the majority of inflammatory tissue macrophages arise from monocytes recruited from blood and that regardless of location, tissue macrophages have similar functions which include surveillance, removal of dead cells and cellular debris, defense against pathogens, promotion of wound healing and tissue remodeling and repair. Eosinophilia in birds rarely occurs but may be associated with parasitism (mites, intestinal parasites, parasites with tissue migration) (Irizaary-Rovira (2004). Acute or chronic inflammatory disease is the predominant cause of monocytosis or heterophilia in pet birds Irizaary Rovira (2004) because monocytes, macrophages and dendritic cells are important hematopoietic cells that play critical roles in defense and in maintaining homeostasis.

Chickens positive for gastrointestinal parasites showed decreased levels of glucose and increased in total protein. Additionally, biochemical studies in a mixed infection show an increase in total protein and aspartate aminotransferase (AST) concentration in serum. Similar reports was mentined by Josué et al. *(*2015) who reported the levels of total protein and globulin were higher in chickens infected with mixed infection by parasites. High levels of total protein in chickens with mixed infection may be related to the immune response against the parasitism (Abdel *et al.,* 2008). It is known that host subjected to parasitic diseases usually activates the humoral and cellular immune responses in order to produce protection against the parasites (Dunbar *et al.,*2005).

6. **CONCLUSION AND RECOMENDATIONS**

Generally,could be concluded that gastrointestinal helminths are one of the major problem in local backyard chicken production. Lesions in the gastrointeste so severe in many cases and this could interfer with digestion and abosortption which in turn could affect the prodcutivity of chicken. Leucocytosis, anemia, dcreased PCV, decreased haemoglobin were another disorders whcih could negativelly contributed to well being of these chicken and as well loss production. All these and others which we didn't evaluate could also negativelly contribute on performance of chicken. The decraese in glucose was another common disordres which could contribute a lot on growth and as productivity.

Based on these conclussions the following recomendations were forwarded

- Screening and treating all positive chicken if possible and if screen all backyard chicken may not be feasible mass and regular deworming
- Improving managment .especialy those that help to braek the life cycle of these parasite
- In addition, proper and adequate nutrition would be provided
- Study with large sample size and area coverage for other parasites should be conducted

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8. ANNEXES

Annex 1: The procedures of blood haemoglobine determination (Ibrahim, 2013).

1. Take 0.1N HCl (1%) into central graduated tube up to mark
2. Suck the blood exactly up to mark 20 (20µl) with the help of sahlis pipette
3. Transfer the blood from pipette to central graduated tube of the hemometer.
4. Mix it well with the help of stirrer or rod and allow it to react for two minute.
5. Make up with distilled water by adding drop by drop until the color matches with the Standard comparator tube and mix well.
6. When the color matches take out and record the values on the side as gm/100ml and or in percentage.
7. Repeat 5 to 6 times and take the average value

Annex 2. The procedures of blood PCV determination (Ibra Bancroft and Gamble, 2002 Ibirahim, 2013).

1. The blood is filled in to a micro hematocrit tube to (3/4th) and sealsit with sealer.
2. Centrifuge the filled hematocrit tube in a hematocrite centrifuge at 12000 rpm for 4-5 Minutes.
3. Read the value (the tube) with hematocrit reader and record the result.

Annex 3. The procedures of the total RBC count (Ibrahim, 2013).

1. Take the blood in to RBC pipette up to 0.5 marks
2. Immediately draw the RBC diluting fluid up to mark 101
3. Rotate the pipette between thumb and other fingers with finger. This gives a dilution of 1:200.
4. Clean the counting chamber of hemocytometer and cover slip
5. Place the cover slip in position over the counting chamber by gentle pressure
6. Expel a drop of blood on to the counting chamber by holding the pipette at an angle of 45º.
7. Allow the hemocytometer for 2-3 min to Settle down the RBC in counting chamber

Counting rules

- Count under 40 x objective under microscope
- Don't C unting the cell touching the bottom and right lines
- Count first from left to right directions and then vice verse
- Counting the cell touching the left and tope lines

Annex 4 Dilusions of common laboratory reagents during study

1 5% Formalin

Formaline, 33%	50.0ml
Distilled water	950.0ml

2 Buffered Formaline (10%)

Sodium hydrogen monobasic	4.5 gm
Sodium hydrogen dibasic	6gm
Formalin, 37%	100ml
Distilled Water	900ml

3. Normal Physiological saline (0.9%)

Nacl	9gm
distilled water	1000ml

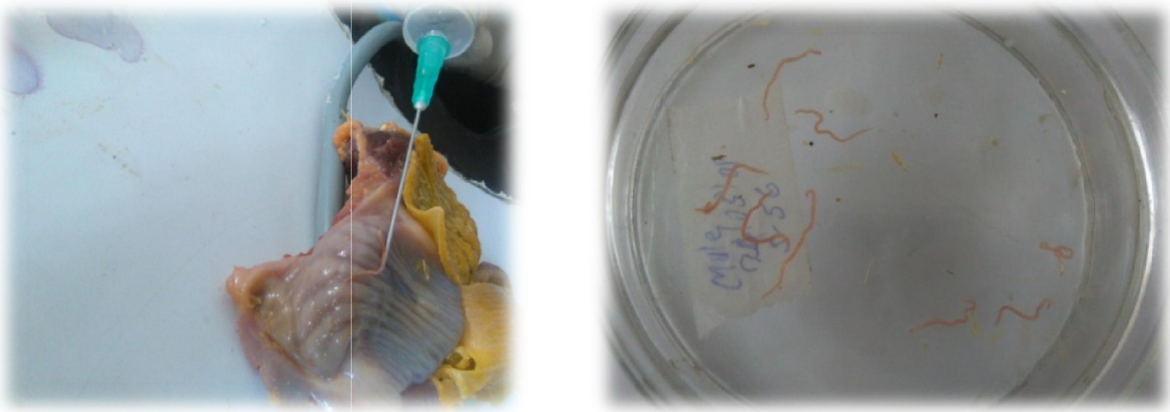
Annex 5: Histopathological procedures (Takulder, 2007)

1. Fixation of tissue by 10% neutral buffered formaldehyde
2. Trimming part of the tissue in a way that the lesion we require be included or not missed and to fit standard histological processing tissue cassettes (5mm thickness).
3. Tissue specimen processing: fixation of tissue by formalin, dehydrating tissue by increasing alcohols concentration, clearing of tissue by xylene, and impregnation of tissue by paraffin wax.

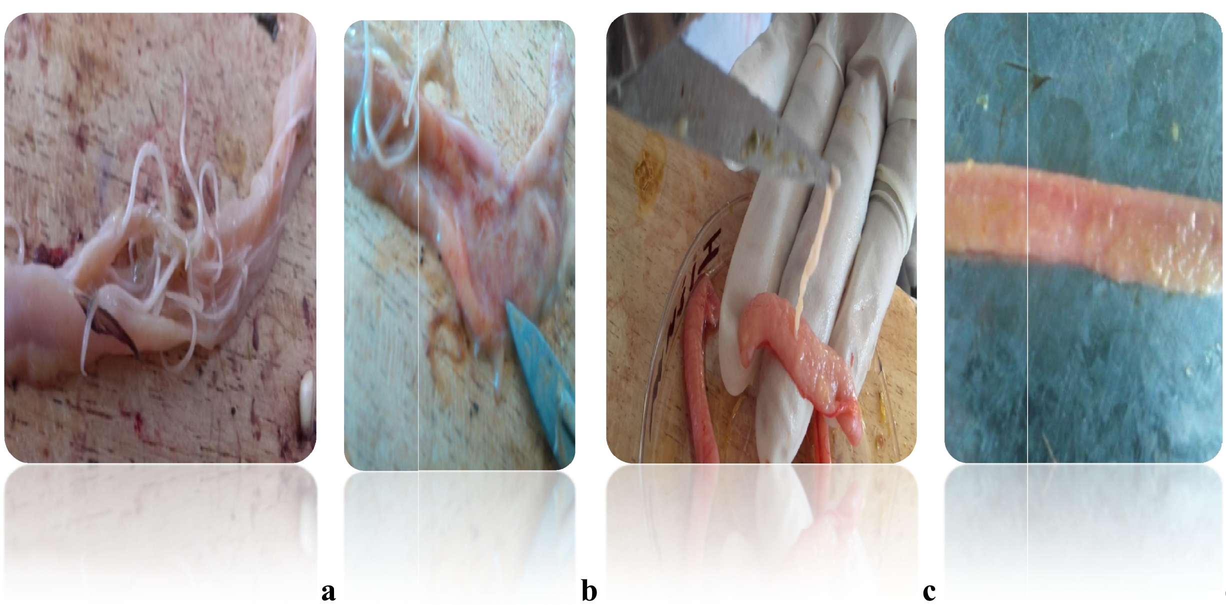
Formalin-I 2hr - ---Formalin-II 2hr - --70% Alcohol 1hr --- 95% Alcohol- 100% Alcohol-I 1hr--- 100% Alcohol-II 2hrs ----- 100% Alcohol-III 2hrs ----- Xylene-I 1:30hrs ------- Xylene-II 1:30hrs ------ Xylene-III 1:30hrs ------ Paraffin-I 2hrs --- Paraffin-II 3hrs.

4. Embedding of processed tissue: impregnated tissue is placed in a mould with their labels and then fresh melted wax (54-60c^{o}) is poured and allowed to settle and solidify.
5. Sectioninig: sectioning of tissue in 3-5 micron thickness and put on water bath to straighten the ribbon, and then adhere on the surface of frost ended and clear slide. Later label and put an Incubator over night.
6. Staining: Hematoxyline eosine stain procedure
   a. Deparaffinize slides in 2 changes of xylene for 5minutes.
   b. Hydrate slides in 3 changes of 100% alcohol each for 3minutes and 1 changes of 95% alcohol for a minute and 1 change of 70% alcohol for 3minutes
   c. Rinse in distilled water until repples disappear from slides.
   d. Place in heamatoxyline (mayer's hematoxline) for 10-15 minutes
   e. Rinse in tap water until water runs clear
   f. Decolorize in 1% acid alcohol, 3-6 qiuck dips. Check differentiation microscopically: Nucleic should be distinct; cytoplasm should be uncolored.
   g. Rinse in tap water until ripples disappear from slides. h. Stain in eosin, 3 dips. i. Rinse in tap water until water runs clear.
   j. Dehydrate in 95% alcohol of 3dips and 100% alcohol, 3 changes eac3minutes.
   k. Clear in 3 changes of xylene for 5 minutes each. l. Mount cover glass with DPX. m. Examination of the prepared slides under the microscope.

Annex 6 : Some pictures of GI helminths Isolated durig study



Adult *Aucaria hamulosa* worm, burrowed in the mucosa(**a**), Adult *Aucaria hamulosa* (red colored worms) isolated from the Mucosa of gizzard (**b**).The keratinized layer of the gizzard was removed during Necropsy.



Intestinal segment harboring large number of *Ascaris galli* (**a**) and associated enteritis characterized by haemorrhagic, edematous and thickened wall **(b)** and *Rellitina Echinobotrida* (c) with associated edematous mucosa and Nodules in the site of attachement **(d).**

Intestinal segment harboring large number of *Ascaris galli*

Picture of Some of the apparently healthy grower and adult chickens (local breeds) of both sexes purchased from open market in the study area and configned in an enclodure (post moprtum room) before sloughter at Addis Ababa University, school of Veterinary Medicine and Agriculture. Age ranging from 7-15 Wks (growers) and above 16 Wks (adults).

Annex 7 : Recording Sheets of lessions, Isolated GI parasites, hemathological and serum biochemical data during research

No	Chicken code	Parasite	Site of infection	Parasite No	Infection	Gross Lesion	Tissue Sample
							-

Hematological parameters of examined scavenging chickens with parasites

Chicken code	**PCV (%)**	**Hb (g/dl)**	**RBC X 10^6/ul**	**WBC X10^3/ul**	**Blood indices**			**Differencial WBC count**					**GI Parasites**
					MCV (fl)	**MCH (pg)**	**MCHC (%)**	**N (%)**	**L (%)**	**M (%)**	**E (%)**	**B (%)**	

Recordig Sheet sof Serum biochemical parameters

Chickens code No	Serum biochemical Variables				
	ALT (U/L)	ALP	AST	Total protein	Glucose