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Study on helminthes prevalence, gross and microscopic characterization of lesions, in scavenging chicken naturally infected by gastrointestinal helminthes in and around Bishoftu, Ethiopia

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Abstract

A study was conducted from November 2016 - May 2017 on eighty free range chickens purchased from Bishoftu local markets, to determine the prevalence and characterization of lesions in parasite positive chicken. Each gastrointestinal tract was spread on dissecting board and separated in different 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. 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 ($x2$=0.278, P>0.05) and among age group ($x2$=0.268, P> 0.05). The frequent gross lesions was recorded and these include necrosis and hemorrhages in gizard, very soft feces containing much mucous exudates in the small intestine, thickened mucosal wall with petechial hemorrhage and thick white pasty mucous in the lumen. After gross lesion characterization tissues were collected into sample bottles containing buffer neutral 10% formalin for microscopic lesion characterization. The fixed tissue sample was trimmed and processed in an automatic tissue processor in different chambers containing different alcohol concentrations cleared in xylene and embedded in paraffin, the block was sectioned at 5 µm, de-waxed, rehydrated and stained using haematoxyline and eosin (H & E) stain. Then allowed for microscopic examination after being mounted by Canada balsam. Microscopic lesions revealed degeneration and necrosis of epithelium and intestinal glands, infiltration of inflammatory cells around the parasite attachments in intestines, excessive tissue damage, hemorrhage, and necrosis in the affected gizzard. Generally, gastrointestinal helminthes are one of the major problems in local backyard chicken production. Lesions in the gastrointestinal so severe in many cases and this could interfere with digestion and absorption which in turn could affect the productivity of chickens.

Keywords: *Bishoftu, Chicken, Ethiopia, Gastrointestinal helminthes, Lesion*

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).

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.

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]) Abrha *et al.* (2014).

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.

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. 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).

Parasitism ranks high among factors that threaten village chicken production. Parasitism causes reduced growth, egg production, emaciation, and anemia 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).

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 the burden of helminthes with Hematological and serum biochemical parameters, in infected scavenging chicken was not so far conducted. Therefore the objective of this study was

- To identify major gastrointestinal helminth species,
- To characterize gross and microscopic lesions that might be caused by these parasites and
- To determine the association of isolated parasites and lesions in naturally infected scavenging domestic chicken.

2. Materials and Methods

2.1. 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)

2.2. 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 proportionally.

2.3. 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.

2.4. Sample size determination and sampling method

The sample size was determined based on the formula recommended by Thrusfield (2005) for simple random sampling method.

$$N = \frac{(1.96)^2 * Pexp * (1-Pexp)}{d^2}$$

Where, N=Sample size required
Pexp=expected prevalence
d = desired absolute precision
1.96^2=z-value for 95% confidence interval

In this formula, the expected prevalence of 97.9% which was reported by (Abebe *et al.,* 1997) in and around Addis Ababa and absolute precision of 95% were considered. Accordingly, a total of 32 chickens were needed. However, for this study a total of 80 chickens were included to increase the precision and accuracy of the result.

2.5. Sample collection and sample processing

2.5.1. 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 formalin for histopathological examination (Bancroft and Gamble, 2002).

2.5.2.. Examination of GI tracts for helminthes

Each gastrointestinal tract was spread on dissecting board and separated in different 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 and the contents was scrapped into Petridish containing 0.9% physiological saline as described by Fatihu (1991). The contents of each section were observed under light microscope for helminthes. Helminthes from each section were isolated and preserved in labeled vials containing formalin. The helminthes were examined and identified as described by Soluby (1982) and Yacob *et al*. (2009).

2.5.3. Tissue processing and examination

After recording the gross changes pieces of intestines from the infected birds were collected and fixed in 10% buffered formalin. 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 were 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.

2.6. Data Analysis

The data collected was entered in to Microsoft Excel spread sheets and analyzed using STATA version13 statistical software's. Descriptive statistics were used to evaluate (frequency and percentages) values. The association of age and sex with helminthes infection in the intestinal tract was assessed Chisquare (X2) test).

3. Results

3.1. Overall prevalence of parasitosis

A total of 80 chickens were 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 helminthes parasites. The frequencies and percentages of individual parasites among the infected chickens are shown in (Figure 1).

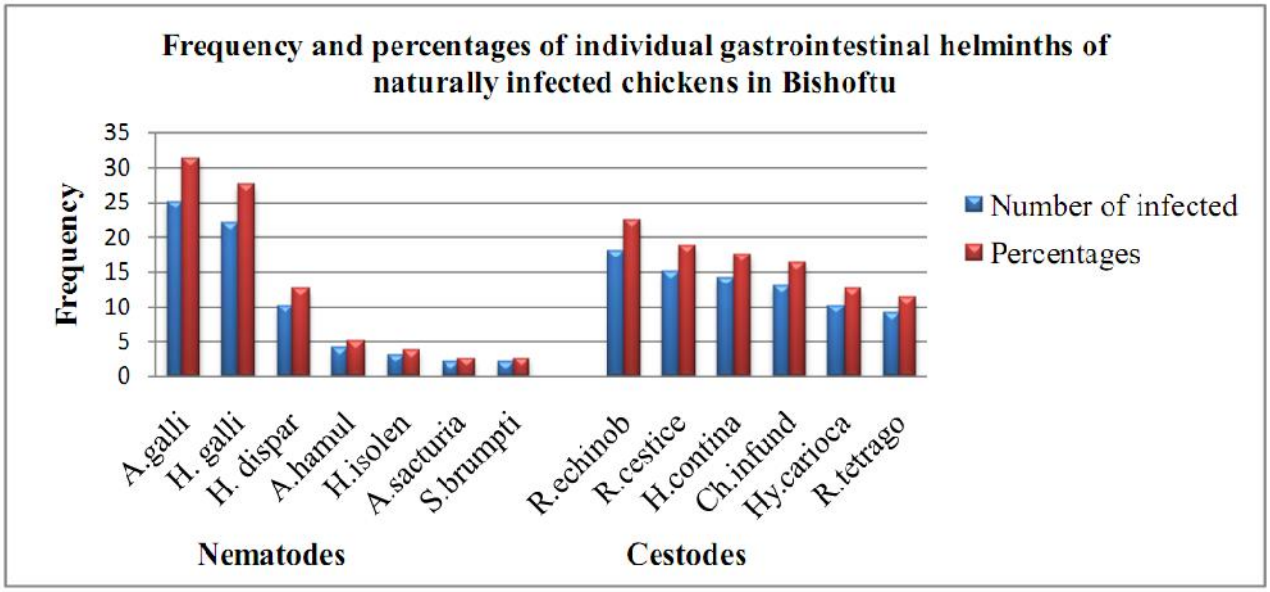

Figure 1:-Species and frequency of gastrointestinal helminthes identified from naturally infected free ranging chickens.

3.2. 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 prevalence and types of parasites identified were presented in table 1 & 2 below.

Table 1: sex and age wise prevalence of individual nematode species

	Number of examined	**No (%) of positive**						
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(0.8)	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(0.8)	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 association in prevalence between individual nematode species and the risk factors (Sex and Age) of the parasite positive chickens($p>0.05$).

Table 2: The prevalence of individual cestode species in relation to host (sex and age) in Bishoftu.

	Number of examined	No (%) of positive					
Variable catagories		***Relliatina. echinob***	***Relliatina Cesticillus.***	***Relliatina tetragonal***	***Choenotenia Infundibulum***	***Hymnolep is Carioca***	***Hymnolepis Continana***
Sex							
Female	39	6 (15.4%)	6 (15.4%)	6 (15.4%)	6 (15.4%)	2 (5.1%) 8	7 (17.9%)
Male	41	12(29.3%)	9 (22%)	3 (7.3%)	7 (17.1%)	(19.5%)	7 (17.1%)
Total	80	18(21.3)	22 (27.5%)	10 (12.5%)	3 (3.7%)	4 (5%)	2 (2.5%)
***x^2* (*p-value*)**		0.12(0.18)	0.45 (0.57)	0.25 (0.3)	0.84 (1.0)	0.52(0.08)	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)	0.86 (1)	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).

Helminthes 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 3).

Table 3: Frequency and percentages of Mixed infection in relation to sex and age with Mixed Infection (N-80)

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

Based on the above result, the gastrointestinal lesions were observed in 25/80 (32%) of examined Chicken of which 18/46 (39%) were from adult and 7/34 (20.6%) from grower and between sexes, male chickens seems to have higher frequency of lesions 13/41 (31.7%) than female 30% (12/39) (Table 4).

Table 4. Frequency and percentages of lesions of local scavenging chickens in relation to sex and age of host.

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)	

Table 5: Frequency and percentages of gross lesions in different intestinal segments of infected chickens by gastrointestinal helminthes.

Variables	No of Examined	No with Lesions	Percentages (%)
Age			
7-15wks (grower)	34	7	20.6
>16wks (Adults)	46	18	39
Sex			
Male	41	13	31.7
Female	39	12	30

*Relliatina cesticillu*s species were seen frequently causing prominent lesions (Greenish or yellowish faeces with a very soft to liquid consistency and containing much mucous exudates) in Jejunum [6 (7.9%)] followed by *Relliatina echinobotrida* in duodenum (Soft to liquid consistency of the intestinal mucosa and nodules) and in and Ilium (Hemorrhagic and sloughed mucosal surface) [5(6.6%)] respectively (Table 5).

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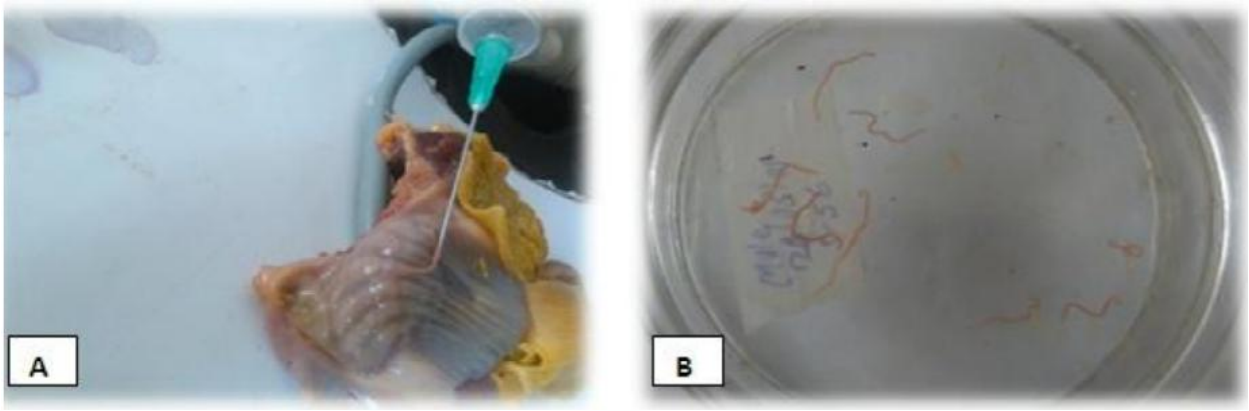

Figure 2: - Adult *Aucaria hamulosa* worm, burrowed in the mucosa of gizzard (**A**), Adult *Aucaria hamulosa* (red colored worms) isolated from the Mucosa of gizzard (**B**).

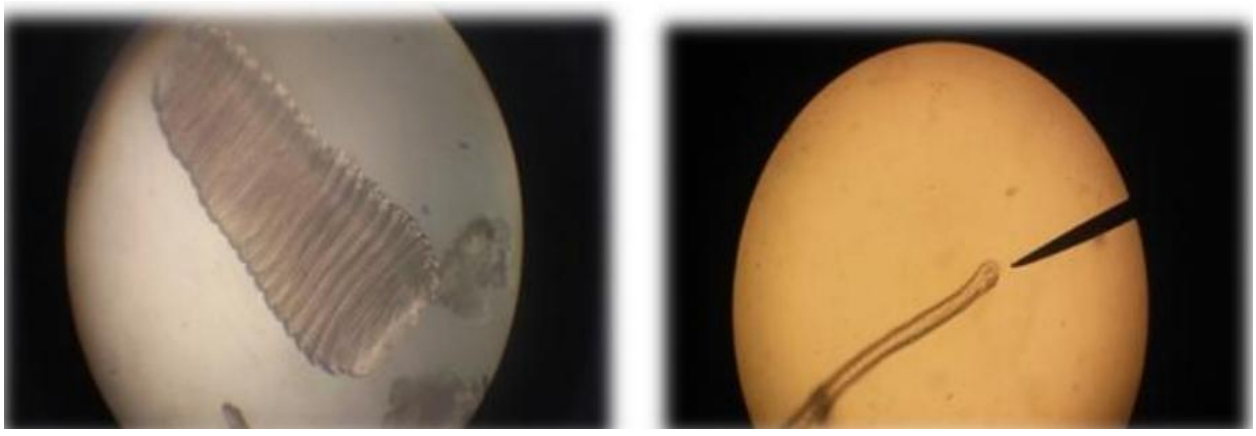

Figure 3:- Immature (Left) and Mature (Right) *Rellietina cesticillus* Collected from the Small intestine of local scavenging chicken (10x).

3.3. Lesions due to gastrointestinal helminthes

3.3.1. Macroscopic lesions

Necropsy examination revealed various gross lesions in gizzard, duodenum, jejunum, ileum, caecum, and large intestine (the details of lesion types and frequency were presented in table 2). The major gross lesions recorded include enteritis characterized by hemorrhagic, edematous and thickened wall. The major gross lesion of gizzard infested *Aucaria hamulosa* is petechial hemorrhage, thickening of the mucosa and hemorrhages into the lumen in some cases (Figure 6).

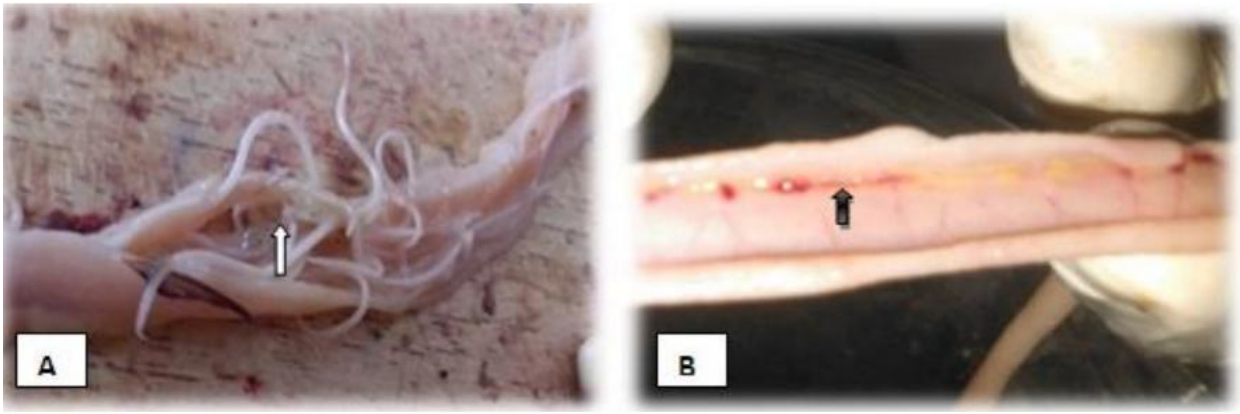

Figure 4: *Ascarida galli* almost blocking the intestine **(A)** and associated enteritis characterized by hemorrhage and thickened intestine (**B**).

Figure 5: *Relliatina .spp* in the in intestine. Note edematous fluid and thickened mucosal surface**.**

Figure 6: Petechial hemorrhages and thickening of mucosa of gizzard affected by *Aucaria hamulosa* (a) and *Ascarida hamulosa i*nduced enteritis characterized by hemorrhagic, edematous and thickened wall (b).

Figure: 7: - *Rellitina Echinobotrida* (**A**) with associated edematous mucosa of the duodenum. Nodules in the site of attachment of *Rellitina Echinobotrida* **(B).**

3.3.2. Histopathological Examination

Total number of 80 tissue samples was 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 *Aucaria*. *hamulosa*, were seen with sever inflammatory nodules around the parasite, mononuclear cell infiltration and sections of the parasites surrounded by inflammatory cells mainly of lymphocytes and plasma cells. In severe cases of gizzard infested with *Aucaria hamulosa* sevre necrosis and removal of cells around the parasite, cellular debris and uniformly esosinophilic edema (Figure 8).

Microscopically, unlike gizzard which is characterized by necrosis and loss of cells around parasites, in the intestines were heavy infiltration of inflammatory cells predominant heterophils and eosinophils (Figure 11).

Figure 8:- 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 9: Picture of jejunum harboring immature (red arrow) and mature *R.cesiticellus* (black arrow), embedded deep into the intestinal mucosa causing severe necrosis of cells leaving cyctic structure around the cestode.

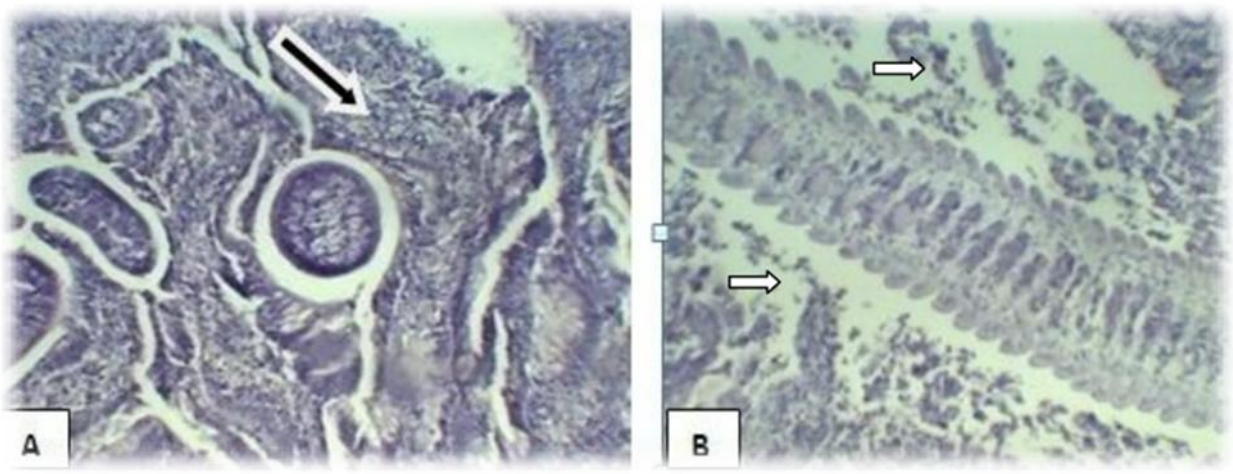

Figure 10: (a) Heavy inflammatory cells (hetrophils) around the parasite. (b) Necrosis of mucosa of duodenum around the parasite and ileum epithelial necrosis.

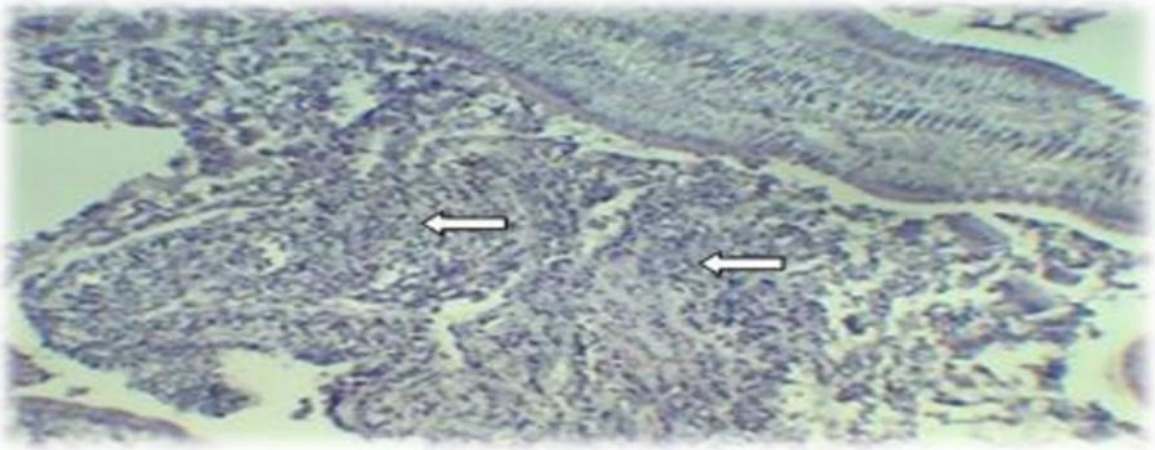

Figure 11: - Heavy inflammatory cells around the cestode. Necrosis of intestinal cells and infiltration of eosinophils into the area.

4. Discussion

High prevalence of gastrointestinal helminthes (95%) in this study was slightly higher than 89.9% of Heyradin *et al*. (2012) from Eastern Shewa Zone. However the result was comparable with 97% of Senyonga (1982) and from ganda, Fatihu *et al*. (1991) 95.7%. These studies indicated also multiple infections with helminthes 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 (Ogbaje *et al*., 2012) or it might also be due to poor bio security, 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 agreement 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 helminthes 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 habits during their incubation periods and most farmers take special care of incubating chickens by giving them feeding like grains and foods remnants and water to compensate for the time spent in incubation Yoriyo *et al*. (2008), and this reduces their chance of picking infections. The male chickens being free increasing their food and mate, thus increasing their chance of picking infections and are therefore more exposed to helminthes infections than females.

In this study no significant difference 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 helminthes species to either sex or age of the host.

The gastrointestinal lesions were observed in 25/76 (32%) of examined parasite positive 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 severe 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 were 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, hemorrhages 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.

5. Conclusion and Recommendations

Generally, It can be concluded that gastrointestinal helminthes are one of the major problem in local backyard chicken production. In the study area, sex and age had no significant influence on the prevalence of poultry helminthes. In general, there was a higher overall prevalence of nematodes and cestodes in local chicken indicating that Helminthes infection is one of the obstacles of poultry production in the study area. Lesions in the gastro intestine were also so severe in many cases and this could interfere with digestion and absorption which in turn could affect the productivity of chicken. Based on the above conclusive remarks the following points are recommended:

- ☞ The farmers in the areas should be aware on the impact of helminthes and a strict measures should be undertaken to control this economically important parasites.
- ☞ In addition, Further large studies may be required to know why this parasites are prevalent in these are and to devise appropriate prevention and control methods, with improved management systems.

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