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Hematological and Serum Changes Associated with Gastrointestinal Helminthes Infection in Naturally Infected Scavenging Chicken in and Around Bishoftu, Ethiopia

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Abstract: A study was conducted on apparently healthy chickens to determine and evaluate change in hematological and serum biochemical parameters associated with gastrointestinal helminthes in apparently healthy chickens purchased from Bishoftu open market. The gastrointestinal helminthes were isolated and characterized. Blood sample was collected from brachial vein in vacationer tube with anticoagulant for hematological examination and also in plain vacationers for serum biochemical analysis. The mean hemoglobin, total erythrocyte count, total leukocyte count and packed cell volume were 11g/dl, 2.67x106/µl, 3.04 x103 / µl, 30.3% and 12g/dl, 3.5 x10/ µl, 2.4 x10/ µl, 36.3%, in naturally infected and non infected negative chicken respectively. 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 difference for the single and mixed infection was statistically significant for aspartate aminotransferase, total protein and glucose. Therefore, it can be concluded that gastrointestinal helminthes are one of the major problem in local backyard chicken production. Sever hematological changes Leucocytosis, anemia, decreased PCV, decreased hemoglobin and another serum biochemical disorders which could negatively contributed to well being of these chicken in loss production and reduced growth rate as well.

Key words: Bishoftu · Chicken · Ethiopia · Gastrointestinal Helminthes · Hematology

INTRODUCTION

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. 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% [1]. 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 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 [2].

Gastrointestinal parasitism in poultry has adverse economic effects on production parameters, so among in backyard or farmyard flocks in comparison to confinements rearing is being adopted in modern commercial farming. Helminthes eg. Round worms (Ascarids), caecal worms (Heterakis), hairworms (Capillaria) and tapeworms having invertebrate intermediate hosts or mechanical vectors play significant roles in poultry production [3]. In addition, processing of each specimen for the desired test with appropriate laboratory procedures and identification of morphological characteristics of gastrointestinal helminthes, their severity and associated hematological and biochemical change should be studied.

In Ethiopia, poor management, nutritional deficiency and poultry diseases are the most important factor in reducing both the chicken's population and their

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productivity [4]. 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 [5]. In and around Bishoftu, studies related to evaluation of hematological and serum biochemical changes in scavenging chicken infected by gastro-intestinal helminthes were not so far conducted. Therefore the objectives of this study was; to identify major species of gastro intestinal parasites harbored scavenging chicken, assess changes in hematological and serum biochemical of infected chickens and to determine the association of isolated parasites and hematological and serum biochemical parameters in naturally infected scavenging domestic chicken.

MATERIALS AND METHODS

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% [6].

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.

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.

Sample Collection and Sample Processing: Blood Collection*:* One ml of blood was collected into vacationer tube with Ethylene diamine tetraacetic acid (EDTA) from wing veins for hematological parameters assessment and 6ml of blood sample was collected in plain vacationers for biochemical analysis, as described by Ross *et al.* [7] and Kavitha *et al*. [8].

Examination of Gastrointestinal Tract (GI) Tracts for Helminthes: Each GI 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 ileo-caeca-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 *et al.* [9]. 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 Soulsby [10] and Yacob *et al.* [11].

Hematological Analysis: The hemoglobin 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 [12]. 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 described by Dein [12].

The Total Red Blood Cell Count (TRBC): The total red blood cell count (TRBC) was performed in 1:200 delusion of blood in Haym's solution. Blood was taken up to 0.5 levels in a RBC diluting pipette. Haym's solution was suck up to 101 marks shifting the blood from the stem to the bulb of the RBC pipette. Mechanically pipette was shaken thoroughly by holding the pipette in between the index finger and thumb. On a clean neubar haemocytometer counting chamber, a drop of diluted blood was placed. The cells were stabilized for 1-2 minutes and total red blood cells in each mm^3 area were counted under low magnification (10x); and the total red blood cells were determined by manual method using hemocytometer according to Dein [12].

Total Leukocyte Count (TLC): Total leukocyte count (TLC) was also determined by taking the fresh blood up to 0.5 level in a white blood cells (WBC) diluting pipette. Glacial accetic acid was suck up to 11 mark shifting the blood from the stem to the bulb of the WBC pipette. Mechanically pipette was ratated gently by holding the pipette in between the index finger and thumb. On a clean neubar counting chamber, a drop of diluted blood was

placed. The cells were stabilized for 1-2 minutes and total white blood cells in each mm area were counted under low magnification (10x); and the total white blood cells were determined by manual method using haemocytometer according to the procedures set by Dein [12].

Hemoglobin Determination: The hemoglobin 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 [12]. The Sahli method is based on converting hemoglobin to acid haematin (brown color) and then visually matching its color 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 color of the diluted blood sample matches the glass standard. The dilution was determined by the Hemoglobin level of the blood sample as described by Philippe [13].

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 Red blood count, Packed Cell Volume and Hemoglobin as described by Ibrahim [14].

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 deferential leukocyte counts using 100×microscopy. Each cell were counted until 100 white cells were counted and the percentage of each WBCs was determined [15].

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°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 and as described by Josue *et al*. [16].

Data Analysis: The data collected was entered in to Microsoft Excel spread sheets and analyzed using STATA version 13 statistical software's. Descriptive statistics were used to evaluate (frequency and percentages) values. The statistical analysis system [17] was used to determine the mean, range and standard deviation of hematological data. The level of the mean values of the infected and uninfected were determined using t-test and a $P<0.05$ [18].

RESULTS

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 (A.hamulosa), Ascaridia galli (A.galli)*, *Hetrakis dispar (H.dispar), Hetrakis gallinarum (H.gallinarum)*, *Hetrakis isolenchae (H.isolenchae)*, *Subulura brumpti (S.brumpti)* and *Allodapa sucturia (A.sucturia*) and 6 cestode species: *Raillietina tetragona (R.tetragona)*, *Raillietina cesticillus (R.cesticillus), Raillietina echinobothrida (R.echinobothrida), Hymenolepis Carioca (H.Carioca, Hymnolepis continana* (*H.continana*) and *Choenetenia infundibulum (C. infundibulum*. 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 (Fig. 1).

Frequency and percentages of mixed infections: 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 1).

Hematological Parameters: The means difference of hematological value between the Parasite negative and parasite positive chickens was significantly different ($p <0.05$), for PCV, RBC, WBC, MCV, Hetrophils, Lymphocytes and Eusinophils (Table 2). Similarly, the means of MCV, MCHC and Lymphocytes were higher and statistically different between growers and adult ($p<0.05$) chickens. But Means difference of hematological value between the sex of chickens was not significantly different ($p >0.05$) (Table 3).

Serum Biochemical Parameters: Between the two (positive and negative) group, the means difference in most biochemical parameters was significantly different

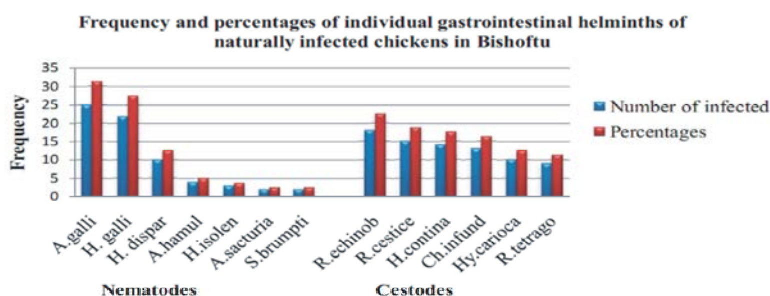

Fig. 1: Species and frequency of gastro-intestinal (GI) helminthes identified from naturally infected free ranging chickens

Table 1: 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)	

Table 2: The mean hematological parameter of positive and Parasite negative chickens

Parameters	Parasite negative chicken (N=4)	Parasite Positive chicken (N=76)	p-value
	Mean ± SE	Mean ±SE	
PCV (%)	36.3±4.35	30.80±0.7	1 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±19.31	16.6±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
Monocyte	1±0.81	2.69±1.69	0.97

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

Table 3: Mean of hematological parameters by sex and age of local chickens

	Sex			Age		
	Female	Male		Grower	Adult	
Parameters	Mean ± SE	Mean ±SE	p-value	Mean ± SE	Mean ±SE	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 (10^6/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±2.7	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.

Table 4: The means of serum biochemical changes of infected and non-infected group

Parameters	Negative Mean ± SE	Positive Mean ±SE	p-*value*
ALT/ul	30.8±0.70	36.25±2.17	0.04
AST/ul	420.9±54.65	341.3±19.36	0.09
ALP/ul	198.8±58.27	552±93.65	0.01
Total protein (g/dl)	7.75±3.32	19.8±1.740.01	
Glucose (g/dl)	245.2±22.3	226.8±8.5	0.01

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

Table 5: 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
Total Protein	3.42±1.74	4.70±0.83	0.04
Glucose	236.29±10.03	227.43±13.67	0.017

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

($p <0.05$), 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 4). In comparison between the single and mixed infection, those chickens positive for multiple gastrointestinal parasites showed decreased levels of glucose and increased in total protein and aspartate aminotransferase (AST) concentration in serum compared to that single parasite infection. Means difference for the single and mixed infection was different for AST, Total protein and glucose ($p<0.05$) (Table 5).

DISCUSSION

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 *et al*. [19] and Ashenafi and Eshetu [20], 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 mean PCV and RBC counts were significantly reduced while WBC, MCV, hetrophil, lymphocyte and eusinohil in positive chicken where increased with a significant difference ($p<0.05$) when compare with the negative chickens. The high infection of intestinal parasites is strongly associated with the development of anemia as they cause malabsorption, nutritional deficiencies and gastrointestinal blood loss [21]. Similar findings were reported from chicken infected by cestode parasites that causes reduction in PCV, RBC count hemoglobin concentration [22]. 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 [22].

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 [23], 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, humeral antibody formation and cell mediated immunity [24, 25]. Eosinophilia in birds rarely occurs but may be associated with parasitism (mites, intestinal parasites, parasites with tissue migration) [24]. Acute or chronic inflammatory disease is the predominant cause of monocytosis or heterophilia in pet birds [24]. 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 mentioned by Josué *et al*. [16], who reported the levels of total protein and globulin was 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 [26]. It is known that host subjected to parasitic diseases usually activates the humeral and cellular immune responses in order to produce protection against the parasites [27].

CONCLUSION AND RECOMMENDATIONS

Generally, it can be concluded that gastrointestinal helminthes are one of the major problem in local backyard chicken production. Hematological changes were so severe in many cases and this could affect the productivity of chicken. Leucocytosis, anemia, decreased

PCV, decreased hemoglobin and another serum biochemical disorders which could negatively contributed to well being of these chicken due to loss of production and reduced growth rate as well.

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