INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print); 2222-5234 (Online)
Website: https://www.innspub.net
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Vol. 27, Issue: 2, p. 76-82, 2025

RESEARCH PAPER OPEN ACCESS

Hematological and biochemical parameters in mono- and associative invasions of domestic chickens by helminths and eimeria in Azerbaijan

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Key words: Hematologic indices, Biochemical indices, Monoinvasion, Associative invasion, Prevalence, Enzyme, Coprological study

DOI: https://dx.doi.org/10.12692/ijb/27.2.76-82 Published: August 06, 2025

ABSTRACT

Hematologic and biochemical studies of birds infected with gastrointestinal parasites (helminths-eimeria) mono- and associatively were carried out in Mountain-Shirvan economic region. For this purpose, the blood of 230 diseased and 20 healthy birds was examined. Serological and biochemical studies were conducted in birds monoinvasion with *Capillaria obsignata*. Serological studies were also carried out in birds infected with helminths and eimeria in associative invasion. In birds associative invasion was noted with parasites *Capillaria obsignata, Raillietina tetragona, Heterakis gallinarum* helminths and primary intestinal parasite - *E. acervulina*. For comparison, serologic studies were performed in healthy birds. In sick birds with monoinvasion, the mean difference of hematologic blood parameters - PCV, RBC, MCV, MCH - was relatively decreased, and WBC, MCHC, neutrophils, lymphocytes and monocytes - relatively increased. The mean difference of hematologic blood parameters in healthy and diseased birds with monoinvasion did not differ significantly in PCV, RBC, WBC, WBC, MCV, neutrophils, lymphocytes and eosinophils ($p > 0.05$). The mean difference of hematologic blood indices in diseased birds with associative invasion - PCV, RBC, MCV, MCH - was sharply decreased, WBC, MCHC was relatively increased, and neutrophils, lymphocytes and monocytes were sharply increased. The mean difference of hematological blood parameters in healthy and diseased chickens with associative invasion was significantly different in PCV, RBC, WBC, MCV, neutrophils, lymphocytes and eosinophils ($p < 0.05$). Mean values of blood biochemical parameters in healthy birds and birds with monoinvasion did not differ significantly ($p > 0.05$). Blood biochemical parameters in birds with associative invasion differed significantly. Differences were observed in ALT, ALP, total protein and glucose ($p < 0.05$). In birds with associative invasion, the mean values of ALT, ALP and total protein were higher, and the mean value of glucose was lower.

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INTRODUCTION

Poultry products are a major source of protein for people worldwide. Bird population density is expected to increase, and in developing countries, they will account for 71.6% of the world's population (Permin *et al.*, 1998). The bird population is projected to be around 16.2 billion (Wanjugu, 2015).

Cases of poultry infected with helminthic pathogens have been reported in poultry farms around the world and are spreading rapidly, causing chick mortality and serious economic losses (Silva *et al.*, 2016; Abede *et al.*, 1997; Kaufmann *et al.*, 2011). Parasitic diseases caused by helminthic pathogens-nematodes, trematodes and cestodes of chickens-are of great importance in the pathology of birds. 60% of these pathogens that cause food refusal and pathological processes in the gastrointestinal tract belong to the nematode class (Puttalakshmamma *et al.*, 2008*;* Beynon *et al.*, 1996).

Researchers note that infection of chickens in backyard farms occurs under conditions of environmental contamination with parasite eggs and larvae (Chauhan *et al.*, 2007; Marizvikuru *et al.*, 2011). Helminthoses in free-range chickens in rural areas are widespread and sometimes occur in subclinical form (Magwisha *et al.*, 2002).

The researchers note that helminthoses in birds are more often found in association with primary intestinal parasites, eimeria, and the species composition of the pathogens is enriched and covers a wide range of areals (Coroian *et al.*, 2024; Nnadi *et al.*, 2010; Jajere *et al.*, 2018).

Associative infestations caused by the endoparasites eimeria and helminths in domestic chickens cause more significant economic damage to the poultry industry. The consequences of these infestations lead to serious problems in poultry production, such as reduced egg production and weight gain of domestic hens, feed refusal and mortality of young chickens. It is noted that the spread of associative invasions caused by gastrointestinal helminths (cestodes, nematodes, trematodes) and eimeria pathogens causing endoparasitic invasions in birds is more intensive (Puttalakshmamma *et al.*, 2008).

Parasitic diseases in domestic chickens are spreading intensively in various regions of the Republic of Azerbaijan, causing the death of young birds. Research is being conducted to study parasitic diseases of domestic chickens in order to identify existing problems in poultry farms and to prevent them.

MATERIALS AND METHODS

The studies were conducted on poultry farms of Gobustan, Agsu, Shamakhi and Ismailli districts, which are part of Mountain-Shirvan economic region. Coprological samples were taken from birds of different age groups and the results were analyzed. Coprological samples from 2340 birds of the economic district were examined for helminths and eimeria. Of these, 230 sick and 20 healthy birds underwent hematologic and biochemical investigations. Coprological samples were examined for helminths by Fullerborn and Vishnauskas methods, and for eimeria by Fullerborn-Darling method. To detect sporogoniums, fecal samples were incubated in potassium bichromate solution for 3-5 days. Morphological characteristics of oocysts and sporocysts (shape, size, color, presence or absence of micropyle and its cap) were used to determine the species (Soulsby, 1982). Studies were conducted on 3-4-month-old chickens, 6-month-old birds and chickens older than 8 months.

Hematological analysis

Blood samples were collected from the brachial vein of 250 birds using a sterile 3 ml syringe and a 23 gauge needle. Each blood sample was immediately transferred into a sterile tube containing the anticoagulant, ethylenediaminetetetraacetic acid. Total red blood cell count (TRBC) or erythrocyte count was performed in a 1:200 dilution of blood in Hiem's solution. Differential leukocyte count was determined by preparing blood smears stained with Wright's dye. Hb concentration was estimated by comparing acidic hematine solution with the standard

stained solution found in a Sahl hemoglobinometer. Red blood cell volume (PCV) was measured by standard manual method after centrifugation of a small amount of blood using microhematocrit capillary tubes. Red blood indices, mean erythrocyte volume (MCV) and mean erythrocyte hemoglobin concentrations (MCHC) were calculated from RBC, PCV and Hb respectively (Coles, 1986; Irizaary-Rovira, 2004).

Biochemical analysis

Blood was collected without anticoagulant to determine serum biochemistry. Serum was separated after centrifugation at 3000 rpm for 15 minutes and stored at -20°C until use. Serum alanine aminotransferase/glutamic pyruvic transaminase (ALT/GPT), aspartate aminotransferase/glutamic oxalic-acetic transaminase (AST/GOT) and alkaline phosphatase (ALP) activities were measured according to the manufacturer's instructions.

Data analysis

Statistical Analysis System (SAS, 2000) was used to determine descriptive statistics of the mean, range and standard deviation of hematologic and serum biochemical data. Mean data were analyzed by comparing with the reference interval value.

RESULTS

Helminth fauna of birds surveyed in mountainous, foothill and plain landscapes of Mountain-Shirvan economic region consisted of 7 species. Nematode fauna of birds consisted of 5 species belonging to 5 genera. Nematode fauna of domestic chickens in the economic region consisted of 5 species - *Ascaridia galli, Capillaria obsignata, Heterakis gallinarum, Trichostrongylus tenuis* and *Syngamus trachea*, and was recorded with high intensity and intensity in foothill and plain landscapes. In domestic chickens, 1 species of the cestode *Raillietina tetragona* and 1 species of the trematode *Echinostoma revolutum* were found in the helminth fauna of birds. Helminth invasion of birds was observed in associative form. Of 2340 coprological samples examined in the economic region, 1860 birds were found to be infected with helminths and eimeria (İ.E. 79.5%). Monoinvasion with helminths or eimeria was noted in 480 birds. Of the 480 birds, in 215 (480/44.8%) were noted with *Capillaria obsignata* monoinvasion and in 86 (265/32.5%) with eimeria invasion. The most intense pathogens of associative invasion were -capillaria + eimeria (1047/56.3%), capillaria + raillietina + eimeria (851/45.8%), capillaria + heterakis + syngamus + eimeria (692/37, 2%), heterakis + syngamus + trichostrongylus + eimeria (771/41.5%), trichostrongylus + heterakis + eimeria (811/43.6%), ascaridia + trichostrongylus + eimeria (447/24.0%), raillietina + ascaridia + eimeria (731/39.3%) (Fig. 1). In birds associatively infected with helminth infection has been observed with the primary intestinal parasite *Eimeria*. The dominant species of *Eimeria* was parasite *E. acervulina*.

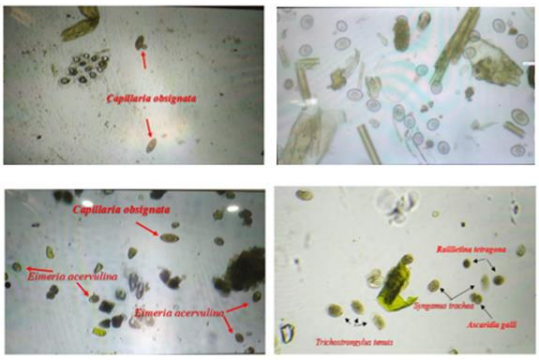

Fig. 1. Helminth and eimeria eggs in coprological samples

Hematological and biochemical parameters are important diagnostic tools in assessing the degree of helminth and intestinal protozoa invasion of birds, and contribute to the effectiveness of treatment. In toxic liver damage, changes in enzyme activity in serologic samples before the appearance of clinical signs are of great importance for early detection of the disease. The prevalence of blood indices in birds infected with helminths and eimeriosis was calculated.

Serological studies were conducted in birds monoinvasion with *Capillaria obsignata*. Serological studies were also carried out in birds infected with helminths and eimeria in associative invasion. In

birds associative invasion was noted with parasites *Capillaria obsignata, Raillietina tetragona, Heterakis gallinarum* helminths and primary intestinal parasite - *E. acervulina*. For comparison, serologic studies were performed in healthy birds.

In sick birds with monoinvasion, the mean difference of hematologic blood parameters - PCV, RBC, MCV, MCH- was relatively decreased, and WBC, MCHC, neutrophils, lymphocytes and monocytes - relatively increased.

Table 1. Hematological parameters of domestic chickens infected with mono- and associative invasion

Parameters	Healthy (n=10)	Monoinvasion (n=65)	Associative invasion (n=65)
	Mean ± SE	Mean ± SE	Mean ±SE
PCV (%)	35.8±4.52	34,7±4,01	31.24±0.7
RBC X 10^6/ul)	3.31±0.03	3,11±0,01	2.41±0.05
WBC X 10^3 /ml)	2.14±0.19	2,56±0,23	3.01±0.07
MCV (fl)	82.5±18.16	81,7±17,21	16.3±18.64
MCH (pg)	43.4±5.21	42,1±4,96	38.13±7,9
MCHC (%)	33.38±4.74	33,95±4,31	36.39±5.78
Netrophils (%)	7.63±3.17	8,45±4,02	13.85±6.9
Lymphocyte (%)	31.48±4.33	32,87±4,90	41.7±5.92
Monocyte (%)	1.05±0.79	1,15±0,16	2.61±1.53
Eosinophil (%)	0.61±0.34	0.96±0.47	9.23±2.68

Table 2. Mean hematologic parameters by age groups of chickens

Parameters	Age group	
	4 months (Mean ± SE)	8 months and older (Mean ±SE)
PCV (%)	30.6±0.82	31.4±1.08
Hb (g/dl)	11.1±0.39	11.1±0.26
RBC (10^6/µl)	2.62±0.09	2.84±0.09
WBC (10^3/µl)	2.99±0.11	3.2±0.11
MCV (fl)	121.4±3.59	110.9±2.8
MCHC (%)	37.5±0.91	35.16±0.83
Heterophils (%)	13.8±1.27	13.4±0.99
Lymphocytes (%)	42.8±0.93	39.9±0.94
Monocytes (%)	2.78±0.31	2.6±0.26
Eosinophil (%)	7.54±2.18	6.82±1.75

The mean difference of hematologic blood parameters in healthy and diseased birds with monoinvasion did not differ significantly in PCV, RBC, WBC, WBC, MCV, neutrophils, lymphocytes and eosinophils ($p > 0.05$). The mean difference of hematologic blood indices in diseased birds with associative invasion - PCV, RBC, MCV, MCH - was sharply decreased, WBC, MCHC was relatively increased, and neutrophils, lymphocytes and monocytes were sharply increased. The mean difference of hematological blood parameters in healthy and diseased chickens with associative invasion was significantly different in PCV, RBC, WBC, MCV, neutrophils, lymphocytes and eosinophils ($p < 0.05$) (Table 1).

Tests were conducted on chickens aged 4 and 8 months and older infected with associative invasion. MCV, MCHC and lymphocyte index were higher in older chickens and statistically significantly different ($p<0.05$). In 4-month-old birds, these indices were not significantly different (Table 2).

In birds with monoinvasion of *Capillaria obsignata*, blood biochemical parameters were investigated. Studies were also carried out in birds infected with associative invasion with helminths and eimeria. For comparison, serologic samples of healthy birds were subjected to biochemical examination. Mean values of blood biochemical parameters in healthy birds and birds with monoinvasion did not differ significantly ($p >0.05$). Blood biochemical parameters in birds with associative invasion differed significantly. Differences were observed in ALT, ALP, total protein and glucose ($p < 0.05$). In birds with associative invasion, the mean values of ALT, ALP and total protein were higher, and the mean value of glucose was lower (Table 3).

Table 3. Biochemical parameters of domestic chickens infected with mono- and associative invasion

Parameters	Healthy (n=10) Mean ± SE	Monoinvasion (n=50) Mean ±SE	Associative invasion (n=50) Mean ±SE
ALT(IU/L)	31.8±0.72	32.3±2.76	36.27±2.19
AST(IU/L)	423.1±54.25	403.2±44.36	342.4±19.27
ALP (IU/L)	198.5±58.17	299.7±66.47	552.1±93.91
Total protein (g/dl)	7.63±3.45	10.1±0.75	19.8±1.62
(Glucose (g/dl)	248.2±23.3	233.4±21.8	216.5±8.7

The comparing blood parameters of birds infected with mono- and associative invasion, glucose levels were sharply reduced in chickens infected with multiple gastrointestinal parasites. An increase in total protein and aspartate aminotransferase (AST) concentration was observed in birds infected with monoinvasion. The mean difference of blood biochemical parameters in birds infected with mono- and associative invasion differed in AST, total protein and glucose ($p < 0.05$).

DISCUSSION

High invasion of domestic chickens by gastrointestinal parasites is closely related to the development of anemia, as they impair nutrient absorption, cause nutrient deficiency and gastrointestinal blood loss (Ejezie *et al.*, 1993). Compared with healthy chickens, leukocyte count, mean erythrocyte volume, heterophils, lymphocytes and eosinophils were significantly elevated ($p < 0.05$), while mean hematocrit and erythrocyte count were significantly decreased. Similar results were observed in cestode-infected chickens in which hematocrit and erythrocyte counts decreased hemoglobin concentration. This pathologic condition may result from the combined effects of vitamin B_{12} deficiency (Aade *et al.*, 2012). An increase in the number of lymphocytes is associated with the consequences of intestinal inflammation. Since the main function of lymphocytes are immunologic response, humoral antibody formation and cellular immunity. In birds, eosinophilia is seen in parasitic diseases (mites, intestinal parasites, parasites with migration into tissues) (Irizaary-Rovira, 2004; Wakenell, 2010). Monocytes, macrophages and dendritic cells play an important role in defense and maintenance of homeostasis, being important hematopoietic cells. Chickens with gastrointestinal parasites have decreased glucose levels and increased total protein. In mixed invasion (helminth+eimeria), biochemical studies show an increase in total protein and aspartate aminotransferase (AST) concentration. The high level of total protein in chickens infected with mixed invasion may be related to the immune response against parasites (Abdel-Fattah *et al.*, 2008). It is known that hosts exposed to parasitic diseases usually activate humoral and cellular immune responses to defend against parasites (Dunbar *et al.*, 2005).

The severity of eimeriosis infection varies depending on the number of oocysts ingested and the immune status of the bird. Some species are easily identified by the size of the oocysts or schizonts (*E. acervulina, E. maxima, E. necatrix* and *E. tenella*) and by the location and appearance of the oocysts in the intestine. The presence of large schizonts in the intestine is pathognomonic for *E. tenella*. The oocysts of *E. brunetti* not only differ from *E. praecox, E. tenella*, and *E. necatrix* in size, but their location in the lower intestine and the appearance of the intestine can be used as reliable indicators. Mild invasions caused by *E. brunetti* can be easily missed if the lower small intestine is not examined. Histopathology of *E. brunetti* reveals schizonts on the fourth day of invasion. (Kaufmann *et al.*, 1996; McDougald *et al.*, 2008).

E. acervulina infection resulted in alpha diversity changes only in the mucosal microbiota, with apparently longer-term effects on the duodenum, while the jejunum was primarily affected in the short term, and the results for beta diversity are consistent with this pattern. Overall, the relative abundance of potentially short-chain fatty acid-producing bacteria tended to be lower in chickens infected with *E.*

acervulina in all four microbiota groups analyzed. The predicted metagenomic functions indicated that pathways associated with the production of short-chain fatty acids, especially butyrate, may be affected by these changes in relative abundance during infection. Further studies of metagenomic function may provide insight into the extent to which duodenal and jejunal bacteria influence chicken health (Campos *et al.*, 2023)

CONCLUSION

According to the findings, gastrointestinal parasites are one of the major problems of domestic chickens in private farms. The hematologic changes are often very serious and affect the productivity of chickens. They cause serious losses in poultry such as mortality, stunting and reduced egg production.

ACKNOWLEDGEMENTS

We would like to thank Hasvet BAKU | International Animal Hospital for his help in conducting and analyzing hematological and blood biochemical studies in mono- and association infestations of domestic chickens.

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