Effect of *Ascaridia galli* infection in some blood parameters in layer hens

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Abstract

The study was conducted to investigate the effects of *Ascaridia galli* in some haematological parameters include, packed cell volume% (P.C.V), Haemoglobin concentration (Hb), total white blood count and differential WBCs count in layer hens by using 75 white lohmann's chicken were randomly divided into 3 groups (25 chicken for each group). The 1st and 2nd groups were infected with 1500 and 250 viable egg/ bird orally respectively, while the 3rd were act as negative control groups (0.1 ml/bird normal saline/ orally). The results of blood examination showed that Packed cell volume% (P.C.V) and haemoglobin (Hb) percentage decreased significant in infected groups, The total leucocytic count and differential WBCs count showed significantly increased in all infected groups.

تأثير الإصابة بطفيلي *Ascaridia galli* على بعض معايير الدم في الدجاج البياض

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الخلاصة

هدفت هذه الدراسة معرفة تأثير الإصابة بطفيلي *Ascaridia galli* في الدجاج البياض على بعض المعايير الدموية (حجم خلايا الدم المضغوطة، خضاب الدم، عدد الخلايا الدم البيض الكلي) من خلال استعمال 75 طير من نوع white lohman، قسمت عشوائيا إلى ثلاث مجاميع متساوية (25 دجاجة/ مجموعة) بعمر 8 أسابيع جرعت المجموعة الأولى 1500 بيضة مصيبة والمجموعة الثانية 250 ببضة مصيبة عن طريق التجريع بالحوصلة مباشرة، وعدت المجموعة الثالثة كمجموعة سيطرة سالبة. أظهرت النتائج وجود انخفاض في مستويات حجم خلايا الدم المضغوطة، خضاب الدم وزيادة عدد خلايا الدم البيض الكلي، والعد التفريقي لكريات الدم البيض، في المجوعتين المصابتين مقارنة بمجموعة السيطرة السالبة.

Introduction

Ascaridia galli is an intestinal helminth parasite belonging to the group of ascarid worms (Phylum Nematoda; Class Secernentia; Order Ascaridida; Family Ascaridiidae). Members of the genus *Ascaridia* are essentially parasitic in birds, and most prevalent in fowl, particularly in chicken and turkey, geese, guinea fowl and a number of wild birds; the principal host manifestly being the chicken (1). Studies have suggested that *A. galli* is the most common nematode in all types of production systems and has a worldwide distribution (2). Infections with *Ascaridia* spp. may cause, in addition to direct losses, reductions in growth rate, weight loss, reduced feed conversion rates and damage to the intestinal mucosa, leading to blood loss and secondary infections (3). Partial or complete obstruction of the intestine, and increased mortality due to secondary bacterial infections(4). The primary damage reduced efficiency of feed utilization, reduced egg production and weight loss are common symptoms in broiler chickens; Young birds are most susceptible, and heavier breeds seem more resistant than the lighter breeds such as

leghorns and white minorcas (5). The life cycle of *A. galli* is direct and the infective stage is eggs harbouring third stage larvae. After ingestion, eggs reach the duodenum and hatch within 24 hours (6), and larvae are released into the lumen of the intestine. The larvae then enter the mucosa of the small intestine and most larvae initiate a histotrophic phase between day 8 to 17 post infection (p.i.) (7). *A. galli* may also play a role in transmission of *Salmonella* infections (8) and avian reo viruses (9) resulting in disease and economic loses. This study was aimed to study effect of *A. galli* infection on some blood parameters in layer hens using different doses.

Materials and Methods

- **Preparation of *Ascaridia galli* eggs:** Adult *Ascaridia galli* female worms were collected from naturally infected chickens according to standard parasitological techniques (3, 10). The uteri of graved female worms were dissected and the eggs were recovered. The eggs were embryonated by incubation in 0.1% (w/v) potassium-dichromate solution at room temperature for 14 days(11). Larvae viability was assessed by observing the larvae spontaneous movement inside the egg after increasing the surrounding temperature. Infective *A. galli* L3 eggs were stored at +4 ◦C until use. On the day of infection another viability test was performed. Only larvae that were well developed, motile and not hatched were accounted as viable and infective. Eggs in the culture were suspended with tap water to get a final volume of 0.5 ml containing the infection dose to be given to each bird. Number of eggs/ml suspension was determined using a McMaster egg counting chamber. The chickens were inoculated via oral gavage at 8 weeks old with an infection doses of 1500 and 250 embryonated eggs per bird.
- **Experimental Design:** Seventy five chickens (8 weeks old) white lohman laying hens were purchased from local layer field and kept in a room 25 m^2 of an experimental animal house, Veterinary Medicine College, Baghdad University, which had free access to water, and the diet was good grounded seeds with supplements vitamins and amino acids with anticoccidial drugs(12). Chickens were divided randomly into three groups (25 chicks each); The hens were orally infected using a plastic Pasteur pipette as described by (10); The 1st and 2nd groups were inoculated with 1500 and 250 infected egg/ bird respectively, 3rd group were inoculated with 0.1 ml of phosphate buffer saline (PBS) orally as negative control group.
- **Blood Parameters:** About 2 ml of blood was collected from each birds (five per group) from wing vein by using a sterile needle and syringe. The blood samples were collected with anticoagulant (EDTA) treated labeled tubes for hematological analysis (Packed Cell Volume, Haemoglobin, White Blood Cell Count and differential WBC count (13).
- **Statistical analysis:** Statistical analysis of means were performed by using statistical package for social science (SPSS, 2008), Version 16, and for determination of a significant differences by using two way analysis ANOVA (14).

Results

- **Packed Cell Volume (P.C.V%): Between group**; The results were showed a significant decrease ($P<0.05$) in the 1st group that infected with 1500 viable eggs/ bird and the 2nd groups at the 3rd and 4th weeks compared with the control group; Also, **within group;** The result was showed a significant decrease ($P<0.05$) in the 1st group during the 3rd and 4th weeks compared with 1st and 2nd weeks, while in the control groups significant difference ($P<0.05$) was recorded 3rd and 4th weeks at. Table (1).

Table (1) Effect of *Ascaridia galli* infection in packet cell volume in layer hens

Weeks / Groups	Mean±SE (%)			
	1	2	3	4
Group 1	25 ± 0.77 A a	25.7 ± 0.77 A a	23 ± 0.44 B b	22.4 ± 0.74 B b
Group 2	25.2 ± 0.8 A a	26 ± 0.63 A a	25.8 ± 1.01 B a	26.4 ± 0.84 B a
Group 3	25 ± 0.42 A a	26.4 ± 0.41 A a	30.4 ± 1.16 A b	31.4 ± 1.07 A b

Different capital letters show a significant difference (P<0.05) between groups. Different small letters show a significant difference (P<0.05) within group. SE= Standard error. N= 5 animals each group. G1= infected with (1500 vaible egg/ bird) G2= infected with (250 vaible egg /bird) G3= Control negative.

- **Hemoglobin (Hb) (g/dl): Between groups**; the result was showed a significant decrease (P<0.05) in the 1st group that infected with 1500 viable eggs/ bird compared with the control group at the 3rd and 4th week; While **within group,** there was no significant difference (P>0.05) recorded. Table (2).

Table (2) Effect of *Ascaridia galli* infection in hemoglobin in in layer

Weeks / Groups	Mean±SE (%)			
	1	2	3	4
Group 1	8.7 ± 0.41 A a	8.88 ± 0.34 A a	7.46 ± 0.25 B a	7.8 ± 0.47 B a
Group 2	8.76 ± 0.43 A a	8.68 ± 0.42 A a	8.42 ± 0.33 AB a	8.14 ± 0.4 AB a
Group 3	8.66 ± 0.24 A a	9.52 ± 0.52 A a	9.82 ± 0.56 A a	9.82 ± 0.56 A a

Different capital hens etters show a significant difference (P<0.05) between groups. Different small letters show a significant difference (P<0.05) within group. SE= Standard error. N= 5 animals each group. G1= infected with (1500 vaible egg/ bird) G2= infected with (250 vaible egg/ bird) G3= Control negative.

- **Total white blood cells count:** The total white blood cells of infected and control groups was showed an irregularity of the means during the period of the study. **Between groups,** a significant difference (P<0.05) was recorded in the 1st and 2nd groups at the 2nd, 3rd and 4th weeks compared with the control group but no significant difference (P>0.05) was found between 1st and 2nd groups at the 3rd week **Within group;** A significant increase (P<0.05) in the 1st and 2nd groups at 2nd, 3rd, 4th weeks compared with the first week. Table (3).

Table (3) Effect of *Ascaridia galli* infection in total white blood cells count in layer hens

Weeks / Groups	Mean±SE (%)			
	1	2	3	4
Group 1	12520 ± 568.7 A a	16940 ± 302.7 C b	25480± 373.4 B b	33880± 1059.9 C b
Group 2	12440 ± 694.7 A a	15140 ± 314 B b	19090± 360.7 B b	26760 ± 892.2 B b
Group 3	13320 ± 528.6 A a	12820 ± 477.9 A a	13440 ± 647 A a	13620 ± 395.5 A a

Different capital letters show a significant difference (P<0.05) between groups. Different small letters show a significant difference (P<0.05) within group. SE= Standard error. N= 5 animals each group. G1= infected with (1500 vaible egg/ bird) G2= infected with (250 vaible egg/bird) G3= Control negative.

- **Lymphocytes: Between groups;** A significant increase (P<0.05) was recorded in the 1st group at 3rd and 4th weeks compared with the 2nd and control groups; While **within group**; A significant increase (P<0.05) was recorded in the 1st group at the 3rd and 4th weeks compared with the 1st and 2nd weeks, while no significant difference (P>0.05) was found in the 2nd and control groups. Table (4).

Table (4) Effect of *Ascaridia galli* infection in lymphocytes in layer hens

Groups \ Weeks	Mean±SE (%)			
	1	2	3	4
Group 1	52.6 ± 1.91 A a	53.4 ± 2.27 A a	60 .2 ± 1.77 C b	62.4 ± 1.12 B b
Group 2	52.4 ± 1.28 A a	53.6 ± 2.27 A a	57 .4 ± 2.01 B a	54.8 ± 0.96 A a
Group 3	54.2 ± 1.62 A a	51.2 ± 1.46 A a	51 ± 0.94 A a	49.4 ± 2.60 A a

Different capital letters show a significant difference (P<0.05) between groups. Different small letters show a significant difference (P<0.05) within group. SE= Standard error. N= 5 animals each group. G1= infected with (1500 vaible egg/ bird) G2= infected with (250 vaible egg/bird) G3= Control negative.

- **Heterophils:** Table (5) was showed a significant increase (p<0.05) in the 1st and 2nd groups that infected with 1500 viable eggs/ bird and 250 viable eggs/ bird respectively compared with negative control group at the 2nd, 3rd and 4th weeks. **Within group**; A significant difference (p<0.05) was recorded in 1st and 2nd groups at 2nd, 3rd and 4th weeks. compared with first week, but no significant difference (p>0.05) was found in the negative control group.

Table (5) Effect of *A*scaridia *galli* infection in heterophils percentage in layer hens

Groups \ Weeks	Mean±SE (%)			
	1	2	3	4
Group 1	27.4 ± 1.16 A a	41.4 ± 1.60 C bc	47.8 ± 2.35 C bd	53.2 ± 2.33 C bd
Group 2	27.4 ± 0.67 A a	34 .2 ± 1.93 B b	39 ± 1.51 B b	40.8 ± 1.88 B b
Group 3	27.6 ± 0.92 A a	27.8 ± 0.73 A a	27.2 ± 1.06 A a	25.2 ± 1.01 A a

Different capital letters show a significant difference (P<0.05) between groups. Different small letters show a significant difference (P<0.05) within group. SE= Standard error. N= 5 animals each group. G1= infected with (1500 vaible egg/ bird) G2= infected with (250 vaible egg/ bird) G3= Control negative.

- **Monocytes:** Table (6) was showed a significant increase (P<0.05) in the 1st and 2nd groups at 3rd and 4th week compared with control group; **Within groups** 1st groups was showed a significant increase (P<0.05) at 3rd, and 4th week. while the 2nd group a significant increase (P<0.05) was recorded at 4th weeks only compared with first week of study.

Table (6) Effect of *Asacridia galli* infection in monocytes in layer hens

Weeks / Groups	Mean±SE (%)			
	1	2	3	4
Group 1	5.2 ± 0.37 A a	5.4 ± 0.5 A a	7.6 ± 0.5 C b	8.2 ± 0.37 C b
Group 2	4.6 ± 0.5 A a	5.6 ± 0.5 A a	5.6 ± 0.6 B a	7 ± 0.63 B b
Group 3	5 ± 0.31 A a	4 ± 0.54 A a	4.6 ± 0.5 A a	4.4 ± 0.4 A a

Different capital letters show a significant difference (P<0.05) between groups. Different small letters show a significant difference (P<0.05) within group. SE= Standard error. N= 5 animals each group. G1= infected with (1500 vaible egg/ bird) G2= infected with (250 vaible egg/bird) G3= Negative control.

- **Eosinophils:** The result **between groups**; was showed a significant increase (P<0.05) in the eosinophils in the 1st group at 2nd, 3rd and 4th weeks compared with the control group while no significant difference (p>0.05) was recorded between the 2nd and control group at 2nd, 3rd and 4th weeks; **Within groups**; A significant increase (P<0.05) was recorded in 1st group at 2nd, 3rd and 4th weeks, and in the 2nd group at the 3rd and 4th week compared with first week. Table (7).

Table (7) Effect of *Ascaridia galli* infection in eosinophils in layer hens

Weeks / Groups	Mean±SE (%)			
	1	2	3	4
Group 1	1.2 ± 0.37 A a	2.4 ± 0.24 B b	4.6 ± 0.5 B b	6 ± 0.54 B b
Group 2	1.4 ± 0.4 A a	2.4 ± 0.40 AB ab	3.2 ± 0.37 AB bc	5.6 ± 0.4 AB bd
Group 3	1.4 ± 0.24 A a	1.2 ± 0.2 A a	1 ± 0.31 A a	0.6 ± 0.24 A a

Different capital letters show a significant difference (P<0.05) between groups. Different small letters show a significant difference (P<0.05) within group. SE= Standard error. N= 5 animals each group. G1= infected with (1500 vaible egg/ bird) G2= infected with (250 vaible egg/bird) G3= Control negative.

- **Basophiles:** Table (8) was showed a significant increase (P<0.05) between groups only in the 1st group at 4th weeks only compared with control group. **Within group**; Also the 1st group was showed a significant increase (P<0.05) at 3rd and 4th weeks, while other groups were showed no significant difference (P>0.05) compared with the first week.

Table (8) Effect of *Ascaridia galli* infection in basophils in layer hens

Weeks / Groups	Mean±SE (%)			
	1	2	3	4
Group 1	0.4 ± 0.24 A a	0.6 ± 0.20 A a	1.6 ± 0.24 A b	1.8 ± 0.37 B b
Group 2	0.4 ± 0.20 A a	0.4 ± 0.24 A a	1 ± 0.31 A a	0.8 ± 0.4 AB a
Group 3	0.6 ± 0.42 A a	0. 4 ± 0.24 A a	1 ± 0.31 A a	0.4 ± 0.24 A a

Different capital letters show a significant difference (P<0.05) between groups. Different small letters show a significant difference (P<0.05) within group. SE= Standard error. N= 5 animals each group. G1= infected with (1500 vaible egg/ bird) G2= infected with (250 vaible egg/bird) G3= Control negative.

Discussion

The fluctuation in hematological values of avian blood are normal phenomenon and in most instances the variations maybe depend on the physiological status of the birds (15). Only a little information is available in the literatures concerning the evaluation of hematologic alterations in birds infected with *Ascaridia galli* and the infection was caused in low health condition and a severe effect when combined with bacterial infection (16). Ghazi *et al*. (17) they were showed that the direct effect of the infection reduce in the body condition, body weight, adult survived, hatching successes and chick survival. Many studies had been reported that the blood parameters most commonly associated with health of individuals and serological and hematological values could be an important source of information and could provide or support an objective assessment of the health status especially the values of Packed cell volume (P.C.V%), total white blood cells count (WBC) and hemoglobin concentration (Hb) (18). A significant difference in the PCV and Hb values were reported between infected chicken and non infected chicken (19). The results were showed that a significant decrease in packed cell volume in infected chicken. Deka and Borah (20) recorded the same finding in their experiment in chicken and quails infected with *Ascardia galli*, and Matta and Ahluwalia (21). The hemoglobin concentration was showed a significant decrease in all infected groups that agreement with Deka and Borah (20) who recorded a similar observation in their experiment. Matta and Ahluwalia (21) were opined that lowered the hemoglobin concentration in infected birds correlated with the activities of early larval stage of *A.galli* in the process of penetration with resultant destruction of mucosa of small intestine and rupture of blood vessels, or might be due to metabolic disturbance caused by worms rather than direct blood loss (22). Saeed *et al*. (19) were reported that the heavy infection with parasites can lead to blood loss and cause an anemia in the birds. Also; The number of WBCs was increased synchronized with the intensity of infection. Also parasites may appear to be associated with hematological changes and this may be due to immune response in the birds (23). Furthermore, leucocytosis and heterophilia had been reported in parasitic diseases in birds (23, 24). Al-Saffar and Al-Mawla (24) had reported that leukocytosis refer to an absolute increase in total number of white blood cells in circulation caused by inflammation resulting from parasitic infections; that agreement with our results. In the present study the total leukocyte count were showed a significant increase in infected birds,this was in agreement with the finding of Deka and Borah (20) in chickens and quails. The elevation in the total leukocyte count might be due to the increase in heterophils and eosinophils because they reform first defense line against body infection Matta and Ahluwalia (21). Also, Eosinophilia was predominant and very characteristic feature seen in birds infected with parasite specially moderate and heavy infections (24). The haemoglobin percentage showed significant decrease in all infected groups than that control group. The lowered haemoglobin concentration in infected birds was correlated with the activities of early larval stage of *A.galli* in the process of penetration with resultant destruction of mucosa of small intestine and rupture of blood vessels. The lowered in Hb values might be due to metabolic disturbance caused by worms rather than direct blood loss.

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