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Original Article

Comparative Effect of *Ascaridia Galli* Infection on the Two Laying Hen Lines on the Liver Function and Immune Response

Saadoon Ibadi Al-Alwani, W[1 *], Hamle Abid, H[1]

1. Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

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Corresponding Author: wroodsadoon1983@gmail.com

Abstract

Chicken production is quickly rising due to the low associated costs and the capability of poultry to convert nutrients into biological protein along with chicken meat accounting for 30% of all animal protein eaten by humans. Despite advances in poultry production, parasitic illnesses in laying hens remain a problem. Farm birds reared in semi-intensive and free-range systems are more prone to parasite infections due to the absorption of polluted water and food from scavenging behaviors and waste droppings. In this study, the effects of *Ascaridia galli* infection on the immune response and liver function of two laying hen lines are compared, and their infection resistance is determined. In total, 50 laying hens at eight weeks of age were used (25 Lohmann brown-classic and 25 Lohmann lsl-lite), and each line was divided into two groups: an infected group (n=15), which was orally infected with a single dose of 500 *A. galli* embryonated eggs, and a control group (n=10), which was given normal saline. After four and eight weeks, blood was collected from the wing vein to assess the serum's AST, ALT, total protein, and IgY levels. The results demonstrated that the infected Lohmann brown-classic and Lohmann lsl-lite chickens presented significantly increased (P≤0.05) AST, ALT, and IgY, compared to the respective control group. Moreover, Lohmann brown-classic hens presented a significantly increased (P≤0.05) IgY concentration four weeks after infection, compared to Lohmann lsl-lite hens. From our results, it can be concluded that genetic variation plays a crucial role in the immune response against *A. galli*, where the Lohmann brown-classic line was found to be more resistant, compared to the Lohmann lsl-lite line.

Keywords: *Ascaridia galli*, Chickens, Resistance, IgY

1. Introduction

Chicken production is quickly rising due to the low associated costs and the capability of poultry to convert nutrients into biological protein along with chicken meat accounting for 30% of all animal protein eaten by humans. Despite advances in poultry production, parasitic illnesses in laying hens remain a problem. Farm birds reared in semi-intensive and free-range systems are more prone to parasite infections due to the absorption of polluted water and food from scavenging behaviors and waste droppings (1).

Compared to confinement rearing, gastrointestinal parasitism in chickens has a negative economic impact on production metrics in-home or farmyard flocks (2). The frequency of helminthiasis infection in chickens was relatively high, with *Ascaridia galli* infection accounting for 31% of the total (3). Infection with *A. galli* causes a decrease in the rate of growth and weight loss; moreover, this parasite may impair laying chicken productivity by decreasing the rate of growth and egg production while increasing feed conversion ratio (4). *A. galli* can modify the immune response of the host,

making it more susceptible to bacterial infections (5). A high worm load damages the intestinal mucosa of chickens, resulting in blood loss, secondary infection, and sometimes small intestine blockage (6). Parasites may play a significant role in decreasing the output of chickens by influencing the development rate of hens and causing tissue damage (7). *A. galli* parasites were linked to decreased health, welfare, immunity, and reproductive performance in laying hens. These factors cause a decrease in egg production and weight gain in sick laying hens, resulting in severe financial losses for owners. Indirect impacts occur when the immune system's function is suppressed, increasing the risk of subsequent infections.

Furthermore, significant *A. galli* diseases may result in a higher death rate in the flock. Within commercial chicken lines, there has been diversity in *A. galli* genetic resistance and between native breeds and hybrid lines (8, 9). Therefore, this study was conducted to measure the immune response and tolerance of different lines of Lohmann to *A. galli*.

2. Materials and Methods

2.1. Preparation and Maturation of *Ascaridia galli* Eggs

Adult females of *A. galli* were collected from infected chickens. The eggs were extracted from crushed female worms in distilled water using a mortar and pestle and filtered into a beaker with a mesh sieve of 0.01 mm diameters. The sediments were rinsed in a beaker with a 0.5 N sodium hydroxide solution. Then, the eggs were incubated for two weeks at 30°C in a 0.1 % (w/v) potassium dichromate ($K_2Cr_2O_7$) (10).

2.2. Experimental Animals

In total, 50 laying hens aged 8 weeks were used (25 Lohmann brown-classic and 25 Lohmann lsl-lite), and each line was divided into two groups: the infected group (n=15) that were infected with a single dose of 500 embryonated eggs through oral inoculation by using a plastic Pasteur pipette according to Schou and Permin (9), and the chickens of the control (n=10) group were given 1 ml of normal saline.

2.3. Feeding and Management of Chickens

Chickens will be raised according to routine management practices outlined by the National Research Council (11). The temperature was set according to the age of the birds (20-24°C). The light was set according to the age of the birds (9-10 hours/day) using a timer. Feeding was conducted using a commercial ration formulated to meet all the nutrient requirements of the birds. Drinking potable tap water without any drugs or supplements was given ad libitum.

2.4. Blood Samples Collection

Six ml of blood was collected from the wing vein at four and eight weeks after infection by using a sterile needle and syringe left to clot, and then, centrifuged at 3000 rpm for 15 minutes to obtain sera. Following that, each blood sample was used to assay IgY concentration in the serum that was measured by ELISA according to the manufacturer's instructions (12). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were measured using an enzymatic colorimetric method (13). Moreover, serum total protein concentration was measured utilizing the enzymatic colorimetric method using spectrophotometry (14).

2.5. Statistical Analysis

The data were subjected to statistical analysis using the analysis of variance for comparison between the different groups. In addition, a post hoc test was used when appropriate to find the least significant differences between different means groups ($P<0.05$). The Statistical Package for the Social Sciences (SPSS, version 26) was used to perform all statistical analyses.

3. Results and Discussion

The study showed that the incubation period of fertile eggs at 30°C for the development and maturation of egg to larvae was 12 days. The microscopic figures (10× and 40×) represent the maturation stage of *A. galli* egg stages (Figure 1). These results agree with Tarbiat, Jansson (15) who discovered that *A. galli* eggs developed quickly for seven days at 30°C. The increased temperature accelerates and

develops the embryonated eggs. Many studies refer to the infection and development that occurs during the summer and spring when the temperature of the environment becomes elevated. This condition is optimal for embryonation (16). Rahimian, Gauly (17) discovered that the eggs maintained at 20-30°C might grow to the infective L2 stage after 7-21 days of incubation and can stay alive at this temperature for an extended period. On the other hand, the result recorded that the use of potassium dichromate has effects on the extra maturation and development of eggs, compared to H_2So_4, and these results were in agreement with the findings of a study by Sharma, Hunt (16). Due to the pH of this solution, the potassium dichromate has an alkaline medium, while H_2So_4 has an acidic medium. Therefore, potassium dichromate is more effective for egg maturation because the eggs of *A. galli* require an alkaline medium for maturation.

The study monitored infected Lohmann brown-classic and Lohmann lsl-lite chickens that significantly ($P \leq 0.05$) increased AST and ALT enzymes, compared to the non-infected group (control group). Moreover, Lohmann brown-classic and Lohmann lsl-lite chickens significantly ($P \leq 0.05$) increased ALT enzyme, compared to the control group (Table 1). Accordingly, considering the increased activity of AST in hepatocytes and its release with liver damage, blood AST levels typically rise quickly after acute hepatocellular injury, reaching values higher than ALT. Acute hepatic injury caused elevation of AST and/or ALT activities (18). Examination of the infected hens by *A. galli* revealed severe anemia and various gross lesions in the liver microscopically followed by an inflammatory cell infiltration surrounding the portal veins, mainly composed of mononuclear cells, as well as subcapsular hemorrhage and fatty degeneration (19). It has also been observed that *A. galli* infection decreases metabolizable nutritional energy in hens due to the parasites' decreased digestibility (20). Previous research has shown that *A. galli* infection in laying hens may affect their stored energy stores, such as liver cholesterol. A significant *A. galli* burden in chickens was recently revealed to result in continuously reduced lipid reserves, compared to uninfected hens (21). Previous studies concluded that the *A. galli* results in severe pathological changes responsible for changing liver function that cause elevation of liver enzymes.

The results (Table 2) revealed that total serum protein levels were considerably lower in both hen lines, compared to the control group; however, it is not significant. This result was in agreement with Deka and Borah (22). It has been reported that intestinal parasitism in anemic birds causes a significant loss of digestive secretion and mucous production, as well as a lack of efficient protein absorption and utilization in the system, ultimately leading to a significant decrease in serum protein levels (23). A significant loss of tissue protein occurs through leakage into the stomach with loss of digestive secretion and mucus owing to intestinal parasitism in anemic birds, which also results in inefficient protein absorption and utilization process, which may account for the decreased total protein levels (24).

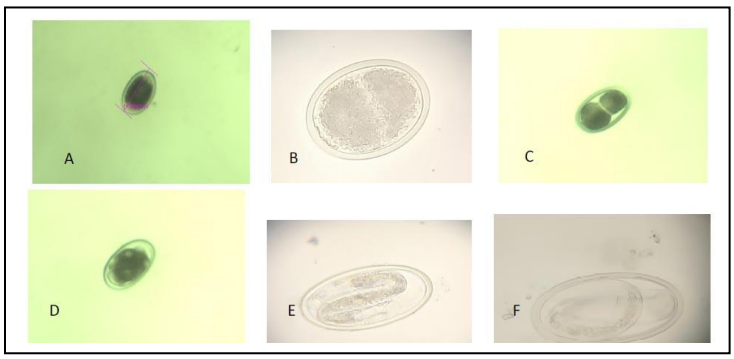

Figure 1. A: Fertilized *Ascaridiagalli* egg (10×), **B and C:** Show egg cells divisions of *Ascaridiagalli* (40 and10×), **D:** Show morula vermiform stage of *Ascaridiagalli* (10×), **E:** Show early embryonated stage (gastrula) (larval 1) of *Ascaridiagalli* (40×), while **F:** Show coiled and motile mature larva (larval 2) of *Ascaridiagalli* (40×)

Table 1. The effect of *Ascaridia galli* infection on the AST and ALT

Parameters	AST/ IU		ALT/ IU	
Layer line Group	**Lohmann brown-classic**	**Lohmann lsl-lite**	**Lohmann brown-classic**	**Lohmann lsl-lite**
Infected	1.42±0.4A	1.28±0.14A	186.02±2.82A	163.6±4.10A
Control	0.84±0.05B	0.78±0.04B	153.3±2.85B	130.6±3.69B

The upper case letters refer to significant differences ($P \leq 0.05$)

Table 2. The effect of *Ascaridia galli* infection on the total protein concentration

Parameters	Total protein g/dl	
Layer line Group	**Lohmann brown-classic**	**Lohmann lsl-lite**
Infected	4.98±021	4.65±0.14
Control	5.13±0.17	4.86±0.3

Non-significant differences

The infected laying hens (Lohmann brown-classic and Lohmann lsl-lite) with 500 embryonated eggs of *A. galli* have an immune response to these infections, and Lohmann brown-classic recorded a significant increment (P≤0.05) in IgY concentrations, compared to their control group at four and eight weeks after infection. Chickens orally infected with *A. galli* eggs acquire cellular and humoral immunity, as shown by the secretion of T-cells helper II-type cytokines and IgY. Norup, Dalgaard (25) regarded *A. galli* infection as an increase in serum antibodies, IgY, and infiltration of both CD4+ and CD8+ positive T-cells at the injection site. Other studies observed that the infected chickens acquire higher levels of specific IgY antibodies against embryonated *A. galli* egg antigens and adult somatic antigens (26). *A. galli* infection raises a serum IgY; however, that may not be enough to protect from infection (25). This finding agrees with Sharma, Hunt (21), who investigated that the concentration of IgY in the serum was higher (P<0.01) in the infected hens than in the control group.

Furthermore, the results revealed that the Lohmann brown-classic line has a higher immune response than the Lohmann lsl-lifeline. Furthermore, brown chicken genotypes have higher parasite resistance than white chicken genotypes, which is a result of their genetic makeup (27), and the genotype background determined the immune responses and level of infection (28). During 80 weeks of experimental *A. galli* infection, researchers looked at substantial differences in particular serum antibody titers in two lines of chickens (25). This might be due to genotypes' different genetic potential, causing them to stimulate IgY more strongly.

On the other hand, it has been shown that the infected chickens' immune systems become more capable of attacking *A. galli* (29). The studies showed that variable chicken genotypes might have different immunological responses to worm infection (25). These are confirmed results of the current study that refer to the Lohmann brown-classic line recorded a significant increase (P≤0.05) in serum IgY concentrations, compared to the Lohmann lsl-lite line and more responses that the IgY was increased significantly (P≤0.05) after four weeks of infection (Table 3). The resistance of laying hen lines may depend on their genetic structure and genetic variation between Lohmann brown-classic and Lohmann lsl-lite. The genetic resistance to nematode loads and antibody responses in chickens is heritable according to research (30).

Table 3. The effect of *Ascaridia galli* infection on the IgY mg/ml in the serum

Time	Four weeks after infection		Eight weeks after infection	
Layer line Group	**Lohmann brown-classic**	**Lohmann lsl-lite**	**Lohmann brown-classic**	**Lohmann lsl-lite**
Infected	4.05±0.32A	2.26±0.24	3.43±0.54A	3.13±0.60A
Control	1.86±0.32B	1.79±0.48	1.71±0.20B	1.83±0.13B

The litters refer to significance differences ($P \leq 0.05$)

The main conclusion that can be drawn from this study is that *A. galli* infection leads to a series of impacts on (but is not limited to) poultry performance and alterations in the biochemical and immune response of chickens. It can be concluded that genetic factors play a crucial role in the resistance to infection and that the Lohmann brown-classic line is more resistant *to A. galli* infection than the Lohmann lsl-lite line.

Authors' Contribution

Study concept and design: H. H. A.
Acquisition of data: H. H. A.
Analysis and interpretation of data: W. S. I. A.
Drafting of the manuscript: W. S. I. A.
Critical revision of the manuscript for important intellectual content: W. S. I. A.
Statistical analysis: W. S. I. A.
Administrative, technical, and material support: W. S. I. A.

Ethics

The study procedures were approved by the ethics committee of the University of Baghdad, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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