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Study of some heamatological parameters of infected pigeons *Columba livia* and coots *Fulica atra* infected with *Hadjelia truncata nematods*

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Abstract

This study was conducted in Al-Najaf Al-Ashraf province, from the beginning of September/ 2019 until January 2020, to identify the effect of the nematode *Hadjelia truncata*, on some heamatological parameters of two types of birds, coot *Fulica atra* and pigeon *Columba livia*. Samples of worms, isolated from birds' gizzards were sent to the Natural History Museum in Baghdad, as they are being studied for the first time, for the purpose of diagnosing samples, while traditional methods were adopted to detect heamatological parameters, for both infected and uninfected birds in order to make comparisons between them, where diagnostics revealed the Infection of *Columba livia* and *Fulica atra* with *Hadjelia truncata*. For both types, the results showed a clear decrease in hemoglobin (Hb), and the volume of packed blood cells (PCV) compared to the control groups, where the values were (8.5 g/ml) and (25.6%) in *Fulica atra*, respectively, while they were in *Columba livia* (15.6 g/ml) and (36.6%), respectively. The increase was clear and noticeable for the WBC numbers of both *Fulica atra* and *Columba livia*, as they were (31.9×10^9/ L) and (1.0×10^9/ L) respectively. For the biochemical indicators, the results showed a decrease in the glucose concentration ratios for *Fulica atra* by (61mg/dl), and an increase for *Columba livia* by (162 mg/dl). The results of the affected birds of both types were in agreement, in the high blood cholesterol concentration, as the *Fulica atra* recorded (338 mg/dl), while the *Columba livia* recorded (233 mg/dl), and the proportions of Ca^{+2} and the AST enzyme were also in agreement in terms of decrease for both the two birds were the values (11.4 mg/dl) and (15 U/L) for the *Fulica atra*, respectively, and (8.5 mg/dl) and (19.2 U/L) for the *Columba livia*, respectively. The results differed in protein and bilirubin concentrations, which showed an increase in *Fulica atra* by (5.7 IU/L) and (0.8 mg/dl), respectively, and a decrease in *Columba livia*, which recorded (3.0 IU/L) and (0.5 mg/dl), respectively.

Keyword: *heamatological parameters, Fulica atra, Hadjelia truncata*

Introduction:

Nematodes are considered one of the most common worms in the world, and they infect humans, animals, insects and even plants, and among these worms is the *Hadjelia truncata* worm, which belongs to the Habronematidae family, and which lives in the gizzard of a number of birds (pigeons, etc.), causing severe disease cases for the affected birds and many economic losses in birds. The life cycle of this parasite has not been clearly studied, and it is believed that the Arthropod plays a role in the infection transmission, being an intermediate host, similar to that to the rest of species of the Spiruroidea family, in its life cycle, and it is possible that the beetles

that these birds feed on are mediator in transferring the infection (1). Infection with this nematode generates various pathological conditions such as weight loss, diarrhea, weakness, molting and a large numbers of host death. The adult parasite settles in the gizzard, and the eggs are released with the bird droppings (2). There are a number of international studies that have dealt with this parasite in some respects such as (3), (4c) and (5), while Iraqi studies have dealt with the spread of the parasite as studies (6) and (7); but this parasite has not been addressed adequately in Iraqi studies in terms of pathology and physiology.

The present study aimed to find out the effect of infection with the nematode *Hadjelia truncata*, on the blood image of some bird species, and to evaluate these blood changes, which included:

Hb, PCV, WBC, Glucose, Cholesterol, Protein, AST, Bilirubin and Ca^{+2} after comparing them to control groups.

Materials and Methods

Collecting bird samples and drawing blood samples:

Ten of coots *Fulica atra*, and 58 of pigeons *Columba livia*, were collected from different areas in Al-Najaf Al-Ashraf province, from September 2019 to January 2020, and blood was drawn from the bird under study from the heart area. Where the blood sample was divided into two sections, the first section was placed in a test tube containing an anticoagulant (Heparin) for the purpose of performing the Hb & PCV tests and all biochemical tests, while the second section was placed in a tube containing EDTA as an anticoagulant in order to perform the examination for counting the number of leukocytes (8) and (9).

Isolation and fixation of nematodes:

The bird was autopsied after being anesthetized with the lethal chloroform, where the abdomen is cut from the bottom of the keeled sternum up to the ribs from both sides, until reaching the shoulder bone, and by raising the chest, the internal viscera is exposed, which includes the gizzard, which is clearly visible, and in the lower left side of body cavity. The gizzard is extracted with the intestine and washed together with running water, then its connection to the intestine is cut off, and it is opened longitudinally for the purpose of examination, the small intestine is isolated and placed in Petri dishes, and it is opened longitudinally for examination to ensure that it is free from any other parasites (8). The gizzard is opened longitudinally with sharp scissors, starting from the contact of the gizzard with the esophagus, passing through the muscle fibers that envelop the gizzard until the middle, then clean with water from all the internal contents inside it, then the horny lining (the koilin layer) lining it is raised, where the worms appear underneath it buried inside the gizzard tissue, it is gently picked up with fine-tip forceps and placed in a Petri Dish filled with normal saline, to be washed and got rid of the stuck materials to it, then it is transferred to a tube, to be fixed using ethyl alcohol at a concentration of 70% and glycerin, to preserve the worms, which were ten placed in Lactophenol on a hot plate, under a temperature of (35-40)◦ C, for a period 24 hours to clarify them and give them the appropriate transparency (10).

Results:

Nematodes were diagnosed by the experts of the Natural History Museum in Baghdad, where it was found that the birds under study were infected with the nematode *Hadjelia truncata*, which intrudes on the gizzard area, specifically under the koilin layer. The results showed, as shown in Table (1), a decrease in the values of

both hemoglobin Hb and PCV volumes of *Fulica atra* infected with *H. truncata*, compared to the control group, where a decrease in the value of hemoglobin concentration was recorded by (8.5 g / ml), compared to the control group (13.0 g / ml). A decrease in the value of the packed blood cell volume was recorded by (25.6%), compared to the control group that amounted to (28.0%). Whereas, the results revealed an increase in the value of the white blood cell count of the infected *Fulica atra* compared to the control group, where an increase in the value of the white blood cell count was recorded by (31.8×10^9 / L) compared to the control group, which was (2.5×10^9 / L). While the results of the *Columba livia* infected with *H. truncata*, as shown in Table (1), they showed a decrease in the values of both hemoglobin Hb and PCV volume of *Columba livia* infected with *H. truncata* compared to the control group, where a decrease in the value of hemoglobin concentration was recorded by (15.6 g / ml), compared to the control group (16.3 g / ml). Also, a decrease in the value of packed blood cell volume was recorded by (36.6%) compared to the control group (48.1%). Whereas, the results revealed an increase in the white blood cell count of the infected *Columba livia* compared to the control group, where an increase in the white blood cell count was recorded by (1.0×10^9 / L) compared to the control group (0.6×10^9 / L).

Test type	Infected *Fulica atra*	Control *Fulica atra*	Infected *Columba livia*	Control *Columba livia*	Unit
Hb	8.5	13.0	15.6	16.3	g/ml
PCV	25.6	28.0	36.6	48.1	%
WBC	31.8	2.5	1.0	0.6	$\times 10^9$/L

Table (1) Changes in physiological indicators (hemoglobin concentration, packed blood cell volume and total number of white blood cells) for both *Fulica atra* and *Columba livia*

While Table (2) shows a decrease in the glucose concentration value of *Fulica atra* infected with *H. truncata* compared to the control group, where a decrease in the value of glucose concentration was recorded by (61 mg / dl), compared to the control group (156 mg / dl). While the increase was noticeable in the values of cholesterol and protein concentrations, where an increase in the value of cholesterol concentration was recorded by (338 mg / dl), compared to the control group, which reached (266 mg / dl), as well as an increase in the value of protein concentration by (5.7 IU / L), compared to the control group that reached (4.2 IU / L), and a decrease in the value of the AST enzyme concentration was recorded by (15.0 U / L), compared to the control group that reached (U / L 15.3), while we find that the results It recorded an increase in the value of the bilirubin concentration, reaching (0.8 mg / dl), compared to the control group, which reached (0.8 mg / dl). As for the calcium concentration, the results showed a decrease in the value of this concentration compared to the control group, where a decrease in the value was recorded. Calcium concentration of (11.4 mg / dl), compared to the control group that reached (11.9 mg / dl). As for *Columba livia* infected with the parasite, table (2) presented the results of its biochemical parameters, as it was found that the values of both glucose and cholesterol concentrations in *Columba livia* increased infected with *H. truncata*, compared to the control group, where an increase in the glucose concentration value was recorded by (162 mg / dl), compared to control group (156 mg / dl). The increase was noticeable

in the value of cholesterol concentration, as an increase of (233 mg / dl) was recorded compared to the control group (186 mg / dl), while the concentrations of protein, AST, bilirubin and calcium decreased for the affected bird compared to the control group, where a decrease was recorded in the value of protein concentration (3.0 IU / L), compared to the control group (6.1 IU / L). As for the AST enzyme concentration, a decrease was recorded (19.3 U / L) compared to the control group that was (27.3 U / L). The same applies to the value of bilirubin concentration, as it recorded a decrease of (0.5 mg / dl) compared to the control group (2.1 mg / dl), and the calcium concentration value of the affected *Columba livia* decreased to reach (8.5 mg / dl) compared to the control group, which reached (11.2 mg / dl).

Table (2) Changes in biochemical indicators (glucose, cholesterol, protein, AST enzyme, bilirubin and calcium) for *Fulica atra* and *Columba livia*

Test type	Infected *Fulica atra*	Control *Fulica atra*	Infected *Columba livia*	Control *Columba livia*	Unit
Glucose	61	156	162	156	mg/dl
Cholesterol	338	266	233	186	mg/dl
Protein	5.7	4.2	3.0	6.1	IU/L
AST	15	15.3	19.2	27.3	U/L
Bilirubin	0.8	0.4	0.5	2.1	mg/dl
Calcium	11.4	11.9	8.5	11.2	mg/dl

Discussion:

Effects of the nematode *H. truncata* on the under study infected birds:

1- Changes in physiological indicators:

A- Hemoglobin concentration (Hb) and packed blood cell volume (PCV):

The current study revealed a decrease in the value of hemoglobin concentration (hemoglobin) and packed blood cell volume (PCV) in the case of birds being infected with the parasite, compared with the control group, and this is attributed to the emergence of anemia in the bird. This study agreed with what was recorded (11) from the same observation in Japanese quails, when infected with the nematode *Ascaridia galli*, and it was mentioned that the decrease in PCV may be due to the decrease in red blood cell concentrations, per unit volume of blood in infected birds. This result is in agreement with the study (12) and (13) that were conducted on domestic ducks and water birds, where the researchers indicated that the low level of hemoglobin in the blood that accompanies the infected bird is due to the deficiency of the copper and iron component resulting from the participation of the parasitic worm of the food absorbed by the bird. The results also agreed with (14) when studying chickens infected with *A. galli*, and (15) that dealt with the effect of parasitism of the nematode *Aporina delafondi* on the physiological and biochemical parameters for pet pigeons subject to starvation, where she attributed the reason for the decrease in hemoglobin and the volume of PCV, which was accompanied by a decrease in the total number of red blood cells, to a decrease in the promoter factor for the formation of red blood cells (Erythropoiesis) in the host. This result was also in agreement with a study (16), which explained the decrease in the number of red blood cells, hemoglobin concentration and the volume of pressurized cells in *Columba livia* infected with the nematode *Raillietina* spp. to the autolysis of red blood cells as a result of being affected by the toxins released by the nematode inside the intestine, which works to

inhibit the production of red blood cells, which leads to a decrease in both the hemoglobin concentration in the blood and the volume of packed blood cell.

B - The total number of white blood cells:

This study also showed an increase in the values of the total number of white blood cells in the sample of the infected bird, for all the species under study compared to the control group, and these results were in agreement with (17), which indicated, in studying the blood changes in Japanese quails naturally infected with the worm *R. tetragona*, that this increase in the number of white blood cells was a natural response to the host, so that the function of the heterophils cells is to devour foreign bodies entering the host's body through their amoebic movement, and therefore its increase in its effectiveness is related to the increase in its numbers as it is the host's first line of defense. While (18) showed that the net increase in the white blood cell count may be due to the increase in heterophils and eosinophils in the blood. These results were also in agreement with (14), (15) and (19).

2- Changes in biochemical indicators:

A- Glucose concentration in blood serum:

The study showed a significant decrease in the concentration of the glucose level, in the case of parasite infection for *Fulica atra* s compared to the control group. These results were identical to the results of study (16) which revealed a low concentration of glucose in *Columba livia* infected with the nematode *R.* spp. because to the worm parasitizes into the intestine, which causes its inflammation and necrosis, and this, in its turns, leads to a lack of absorption of nutrients, including glucose, as well as the worm's competition with the bird for the food that it eats.

The results of the current study showed an increase in the percentage of blood glucose concentration in the *Columba livia* infected with the worm compared to the control group, making it inconsistent with what was mentioned above from the results of the studies, while the result was consistent with what (15) mentioned, about no change in glucose concentration in groups of *Columba livia* infected with *R*. spp. This is explained by the fact that birds have a storage capacity for ample quantities of sugars in their vesicles, which gives them the ability to maintain a high level of glucose compared to other vertebrates, and this may be a reason for throwing good quantities of glucose into the blood of *Columba livia* under the current study.

B- Cholesterol concentration in blood serum:

The current study showed the high level of cholesterol in the blood of birds in the study and infected with the nematode compared to the control group. Perhaps this is due to the nature of migratory birds in storing large amounts of fat inside their bodies in preparation for migratory trips (20) and the results of this study matched (21) where it was recorded high levels of cholesterol in frog infected with *Waltonella duboisi*. This may be attributed to an increase in the rate of lipolysis as an alternative source of energy due to insufficient absorption of carbohydrates and a decrease in glycogen storage in the infected frog. These results were not in agreement with a study (22) that dealt with parasitism of *A. galli* on quails *Coturnix coturnix*. The reason for this may be due to the worms ’effect on fat absorption.

C- Protein concentration in blood serum:

The current study revealed the high level of protein in the blood of a nematode-infected *Fulica atra* compared to the control group, and in this aspect, the characteristics of migratory birds may play a role in explaining this rise, as migratory

birds usually store quantities of protein in the nutrient stores inside their bodies in anticipation of any emergency during flights migration so that the bird would not starve during the long flight period (23). This result did not agree with (22), which showed a decrease in the percentage of protein in the blood of quails *C. coturnix* infected with *A. galli* compared to the control group, the researchers attributed this decrease to the leakage of tissue proteins in the intestine with the loss of digestive secretions and mucus resulting from intestinal parasitism in anemic birds, as well as the effect of that parasitism on the inefficiency of protein absorption while the results showed a decrease in the concentration of protein in the blood of infected pigeons compared to the control group, and this result was in agreement with previous results the above mentioned, as the study agreed with (24), who attributed the cause of this to poor digestion resulting from infection with the parasite, which leads to malabsorption, and indicated that this is the main reason for the clinical and blood disorder of the birds, and the result also agreed with (15), which stated that the reason for the decrease may be due to the occurrence of a state of deficiency in blood protein due to an increase in intestinal velocity as occurs in diarrhea, which is associated with a rapid loss of protein, while the researchers indicated (25) that nematodes have a limited ability to build proteins and cannot build basic amino acids. Therefore, they depend mainly on their nutrition from the host to obtain them, which negatively affects the host.

D- AST enzyme concentration in blood serum:

The current study indicated a decrease in the concentration of AST enzyme for all birds under study infected with the parasite when compared to the control group. The decrease in the AST enzyme may fall under the category of malabsorption of vitamins that birds suffer due to parasitism, especially vitamin B6, as this vitamin is a catalyst for the work of both AST & ALT enzymes during their interactions (26) and enhances the use of nematodes from vitamin B6 what the study (27) mentioned about the disappearance of larvae *Litomosoides carinii* (Nematoda: Filaroidea) from the blood of infected cotton mice suffering from a lack of peroxidine (Vitamin B6), as their disappearance is due to the lack of enzymes derived from B6 (Essential B6-derivative coenzymes) necessary for the proper metabolism of the parasite.

These results were in agreement with (28) that showed the effect of some nematodes and tapeworms in domestic chickens, while these results were not in agreement with a study (15), while a study (29) added that AST enzyme exists in cells in two forms, one in the mitochondria and the other in the cytoplasm. An increase in the concentration of this enzyme does not necessarily indicate a malfunction in the liver, but rather a breakdown and destruction in its cells that led to the leakage of enzymes from them.

E - Bilirubin concentration in blood serum:

The current study reported an increase in the value of the bilirubin concentration in the blood of infected *Fulica atra* when compared with the control group, and this result agreed with (30) who observed an increase in the percentage of bilirubin and bilverdin in chickens infected with malaria parasites, referring to the cause of the occurrence of hemolysis due to infection, as it was also identical to (31) that revealed a high level of bilirubin in broilers infected with hepatitis and pericardial syndrome, indicating damage to hepatic cells and biliary obstruction due to infection. High level of bilirubin is usually attributed to diseases related to the liver or diseases that cause retention of bile flow or an increase in the breakdown of red blood cells (29), and since the habitat of the worm parasitism is far from the liver area, so diseases related

to the liver and bile were excluded to be a cause of an increase in bilirubin, and perhaps the parasite's movement and its invasion into deep areas of the gizzard caused hemorrhage and breakdown in some red blood cells as a result this slight increase in bilirubin. The current study reported a decrease in the values of bilirubin concentrations in the blood of pigeons infected with *H. truncata* compared to the control group, and this result did not agree with the results mentioned above.

F- Calcium concentration in blood serum:

The current study showed a decrease in the values of calcium concentrations in the blood of coot and pigeon infected with the *H. truncata* compared to the control group, and perhaps the low level of calcium in the blood of these birds is due to the worm's competition with the bird to absorb nutrients from its blood, which prevents the bird from obtaining sufficient amounts of calcium, especially since the worm often it tends to adjust its calcium levels while dwelling in the host's body as it regulates calcium homeostasis within its bodies through Na^{+1} / Ca^{+2} exchange channels precisely designed to meet physiological requirements, the most important of which is the transfer of calcium ion into cells to be an important regulator, especially for movement from during its influence on the motor neuronal synaptic activity as well as its role in regulating the life span of the worm, cell division and growth, regulation of genetic transcription, muscle contraction, secretion, ions transport, egg formation and others (32) and (33). These results were identical with (28) that showed the effect of some nematodes and tapeworms in local chickens, this is explained by the simultaneous decrease in sodium ions with water due to the loss of water from the body, which leads to decreased extracellular fluid volume.

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