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Alterations of Blood Components in Broiler Chicks Experimentally Infected with *Salmonella* Gallinarum

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Abstract: *Salmonella* Gallinarum is the agent of fowl typhoid in poultry causing significant economic losses in poultry production. The present study was conducted to investigate the effect of experimental infection of one-day-old broiler chicks with locally isolated *Salmonella* Gallinarum strain (the cause of acute fowl typhoid disease) on hematological and biochemical constituents. One hundred and forty broiler chicks -one day old- were randomly divided into two groups. The first group (N=50) was kept as a normal control. It was inoculated with 0.2 ml of sterile saline through crop gavage. The second group (N=90) was infected with isolated *Salmonella* Gallinarum strain at a dose 0.2 ml of sterile saline containing 3×10^8 CFU/ml through the same route. After inoculation, all experimental birds were kept under strict daily observation for recording clinical signs and mortality rates. Five blood samples were collected from each group at the zero time, 2nd, 4th, 7th, 14th, 21st and the 28th day post infection for determination of hematological and serum biochemical parameters. The infected group showed clinical signs (dullness, ruffled feathers, droppings, huddled together, white pasty diarrhea, loss of appetite, decrease in feed intake and depression), mortality rate (24.4%), macrocytic hypochromic anaemia, leuckocytosis, heterophilia, significant hypoprotinemia, hypoalbuminemia, hypoglobulinemia and marked decrease in the serum iron level, in addition to marked increase in the activities of aspartate aminotransferase and alanin aminotransferase and the levels of creatinine and uric acid. In conclusion, the experimental *Salmonella* Gallinarum infection induced acute anaemia, leukocytosis, heterophilia, lymphopenia and alteration in the liver and kidney functions.

Key words: Broiler chicks · Fowl typhoid · Hemogram · Serum biochemistry

INTRODUCTION

Salmonella Gallinarum (S. Gallinarum) is the agent of fowl typhoid in poultry causing significant economic losses in poultry production. Fowl typhoid is a severe and septicemic disease affecting primarily mature chicken and turkey flocks; however there are reports of high mortality in young chicks [1]. These economic losses are represented by high mortality rates in baby chicks, retardation in growth, adverse effect on egg production of infected laying birds and low fertility and hatchability of eggs laid by carriers [1].

Specific *S.* Gallinarum infection is commonly systemic causing sepsis independently of age [2]. Mortality caused by *S*. Gallinarum may be higher than 80% in broilers [3] and may reach almost 100% in inoculated young brown layers [4] and may reach 100% in some infected flocks [5]. Common symptoms are depression, weakness, ruffled feathers [6], weight loss, 50-70% drop in egg production [7] prostration, apathy, drooped wings, loss of appetite, dehydration and greenish-yellow to bloody diarrhea [8]. Fowl typhoid affects primarily chickens and turkeys, but pheasants, quails and guinea-fowl are also susceptible [9].

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Biochemical and hematological changes have been confirmed as useful parameters for detection and identification of avian diseases [10]. Avian salmonellosis has been considered by many previous investigators all-over the world to be of special significance due to its danger to human beings and poultry farms [11]. *Salmonella* Gallinarum produced lesions in chicks, indistinguishable from those associated with pullorum disease [12]. Many hematological changes were reported during the course of acute fowl typhoid infection in broiler chickens, including severe acute anaemia, reticulocytosis and erythrocytes modification [13]. In addition, hepatitis, splenitis, typhlitis, omphalitis, myocarditis, ventriculitis, pneumonia, synovitis, peritonitis and ophthalmitis were observed in acute fowl typhoid [9]. The present work was conducted to investigate the effect of experimental infection of one-day-old broiler chicks with locally isolated *Salmonella* Gallinarum strain on hematological and biochemical constituents.

MATERIALS AND METHODS

This study was carried out according to guidelines for animal experimentation and approved by the Institutional Animal Care and Use Committee, National Research Centre Animal Care Unit, Dokki, Giza, Egypt.

Isolation and Identification of *Salmonella* Gallinarum Strain: It was performed from the intestine and liver of diseased chicks and chickens showing high mortalities, diarrhea, ruffled feathers and anorexia according to Finegold and Martin [14], Quinn *et al*. [15] and Swayne *et al*. [16]. The previous samples were collected from one hundred and twenty eight broiler chicks and layer chickens from private farms located at Sharkia and Dakahlia Governorates. Suspected *Salmonella* isolates were identified serologically using the slide agglutination test in the Laboratories of Ministry of Public Health, Cairo, Egypt, according to Neville and Bryant [17].

Chicks: One hundred and forty of one-day-old Hubbard broiler chicks were used in this experiment. These chicks were put under the required hygienic condition, fed on a balanced ration, supplied with clean water in sufficient quantities and not supplemented with antimicrobial agents until the end of the experimental period.

Preparation of the Inoculum (Cultured Strain): Twenty-four hours pure cultures of identified *S.* Gallinarum were suspended in sterile saline solution using McFarland opacity tube No. 1 [18]. The approximate cell density in this dilution was 3×10^8 CFU/ml according to Freitas *et al*. [6]. One-day-old chicks were crop gavage, each one was inoculated with 0.2 ml of sterile saline containing 3×10^8 CFU/ml [6].

Experimental Design: This experiment was carried out at the experimental Animal unit of the Lab Animal House, National Research Center, Dokki, Giza, Egypt. One hundred and forty -one day old- chicks were randomly divided into two groups. The first group (N=50) was kept as a normal control and each one was inoculated with 0.2 ml of sterile saline through crop gavage. The second group (N=90) was infected with isolated *S.* Gallinarum strain at a dose 0.2 ml of sterile saline containing 3×10^8 CFU/ml through the same route. After inoculation, all experimental birds were kept under strict daily observation for recording clinical signs and mortality rates. Five blood samples were collected from each group at the zero time, 2nd, 4th, 7th, 14th, 21st and the 28th day post infection (dpi) for determination of hematological and serum biochemical parameters. Moreover these chicks were sacrificed for bacteriological examination.

Bacteriological Examination: All necropsied chicks were exposed to bacteriological examination. Moreover, re-isolation and identification of the inoculated *S.* Gallinarum was also performed from the liver and intestine at the 7th, 14th, 21st and the 28th dpi. Identification of the re-isolated *S.* Gallinarum was depending on morphological characters and biochemical properties [14].

Blood Samples: Five blood samples were taken by jugular puncture from each group at the zero time (Before experimental infection), the 2nd, 4th, 7th, 14th, 21st and the 28th dpi. Each blood sample was divided into two portions; the first one was anti-coagulated with tri-potassium EDTA and was used for determination of hematological investigations. The second portion was placed in a plain centrifuge tube for serum separation and used for determination of serum biochemical parameters.

Hematological Evaluation: Erythrogram (Red blood cell counts (RBCs), hemoglobin (Hb), packed cell volume (PCV) and RBCs indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC ((and leukogram (Total leukocytes and its differential leukocyte counts) were performed by the method of Feldman *et al*. [19] using Natt and Herrick solution as diluent [20].

Serum Biochemical Assessment: Total proteins [21], albumin [22], iron [23], alanin aminotransferase (ALT), aspartate aminotransferase (AST) [24], alkaline phosphatase) ALP) [25], uric acid [26] and creatinine [27] were performed by using test kits supplied by Bio-diagnostic, Dokki, Giza, Egypt.

Statistical Analysis: All data were subjected to statistical analysis including the calculation of the mean and standard error of the mean. Significance between data of groups was evaluated by student *t*-test at levels $P > 0.05$ [28] using SPSS (Statistical Package for Social Sciences) version 15 Computer program.

RESULTS

Clinical Signs: Clinical signs following the experimental infection with *S.* Gallinarum were represented by dullness, ruffled feathers, droppings, huddled together, white pasty diarrhea, loss of appetite, emaciation and depression.

Mortality Rate: The mortality rate was 24.40% among experimentally infected chicks (Table 1), while no abnormal signs or gross lesions were observed in normal control chicks during the experimental periods.

Bacteriological Examination: *Salmonella* Gallinarum was isolated from chicks after the 7th, 14th, 21st and the 28th dpi.

Hematological Findings: At the 2nd dpi, no significant changes were detected in the erythrogram of infected chicks compared to the control group. RBCs count, Hb concentration and MCHC significantly decreased in the infected group from the 4th dpi until the end of the experiment, while there was a significant increase in the PCV% in the infected group at the 4th and 7th dpi. However, there was a highly significant increase in MCV in the infected group started from the 4th dpi till the end of the experiment. In addition, at the 7th dpi MCH significantly increased in the infected group compared to the control one (Table 2).

Table 1: Mortality rate in normal control and infected chicks with *S.* Gallinarum during 28 days post infection

	Mortalities after S. gallinarum infection (days)												
Groups	12h	1	2	3	4	5	6	7	8-14	15-21	22-28	Total	Mortality rate
Infected (N=90)	0	0	5	3	2	4	0	2	4	1	1	22	24.4%
Control (N=50)	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2: Hematological parameters of normal control and infected chicks with *Salmonella* Gallinarum during 28 days post infection. (Mean±SE)

		Periods (Days)						
Parameters	Groups	0	2	4	7	14	21	28
Red blood cell Counts (×106 /µl)	Control	1.98±0.01	2.05±0.05	2.23±0.10	2.37±0.10	2.35±0.12	2.42±0.16	2.29±0.12
	Infected	2.10±0.07	1.92±0.03	1.80**±0.02	1.71**±0.03	1.65**±0.04	1.62**±0.02	1.88*±0.02
Packed cell volume %	Control	27.73±0.25	28.10±0.26	30.08±0.14	30.78±0.23	32.92±0.14	32.76±0.26	33.02±0.12
	Infected	27.84±0.22	28.24±0.19	31.40**±0.21	32.04**±0.16	32.76±0.22	32.66±0.30	33.16±0.09
Hemoglobin (g/dl)	Control	9.07±0.21	9.99±0.10	10.02±0.08	11.09±0.16	11.68±0.11	12.45±0.24	12.31±0.14
	Infected	9.52±0.21	9.78±0.22	9.07**±0.12	9.26**±0.12	8.71**±0.19	8.82**±0.13	10.30**±0.19
Mean corpuscular volume (fl)	Control	140.07±1.67	137.23±3.40	136.01±5.53	130.85±5.69	141.46±6.47	137.54±9.32	145.72±7.10
	Infected	132.95±5.09	146.96±2.71	174.50**±1.44	187.09**±2.68	198.82**±5.40	202.25**±3.15	176.24**±1.47
Mean corpuscular Hemoglobin (pg)	Control	45.81±1.00	48.79±0.88	45.39±2.36	47.07±1.60	50.23±2.41	52.04±2.60	54.31±2.51
	Infected	45.40±1.48	50.97±1.78	50.39±0.48	54.08**±0.81	52.79±1.24	54.69±1.45	54.80±1.33
mean corpuscular hemoglobin concentration (g/dl)	Control	32.74±0.97	35.58±0.56	33.31±0.39	36.03±0.41	35.50±0.27	38.03±0.78	37.29±0.37
	Infected	34.22±0.85	34.68±0.98	28.88**±0.26	28.92**±0.45	26.59**±0.63	27.04**±0.60	31.08**±0.56
Total leukocytic counts (×103/µl)	Control	18.42±0.42	19.22±0.14	20.50±0.27	20.86±0.38	19.84±0.38	20.38±0.50	21.81±0.38
	Infected	18.71±0.31	34.30**±0.34	41.29**±0.69	47.37**±0.37	27.33**±0.22	22.31*±0.42	24.93*±0.42
Heterophils (×103/µl)	Control	4.57±0.16	4.74±0.14	5.03±0.10	5.07±0.16	4.80±0.15	4.74±0.12	5.26±0.11
	Infected	4.54±0.25	21.37**±0.38	29.13**±0.11	35.14**±0.30	11.19**±0.14	6.74*±0.22	6.91±0.22
Eosinophils (×103/µl)	Control	0.13±0.01	0.12±0.01	0.12±0.01	0.13±0.01	0.11±0.01	0.11±0.01	0.12±0.01
	Infected	0.13±0.01	0.11*±0.01	0.11*±0.01	0.11*±0.01	0.11±0.01	0.12*±0.01	0.12±0.01
Lymphocytes (×103/µl)	Control	11.39±0.25	11.65±0.20	12.82±0.21	13.12±0.14	11.40±0.20	13.00±0.24	14.03±0.23
	Infected	11.78±0.21	9.72*±0.34	9.19*±0.22	9.19*±0.22	9.88*±0.18	12.96±0.38	14.36±0.21
Monocytes (×103/µl)	Control	1.17±0.09	1.36±0.03	1.75±0.04	1.72±0.03	1.44±0.02	1.63±0.03	1.64±0.03
	Infected	1.33±0.10	1.32±0.07	1.72±0.03	1.71±0.02	1.69*±0.03	1.63±0.03	1.63±0.03

SE = Standard error. = * Significant at $P \leq 0.05$. ** = Highly significant at $P \leq 0.01$. Number of chicks = 5

Table 3: Serum biochemical changes of normal control and infected Broiler chicks with *Salmonella* Gallinarum during 28 days post infection. (Mean±SE)

		Periods (Days)						
Parameters	Groups	0	2	4	7	14	21	28
Total proteins (g/dl)	Control	3.14±0.12	3.55±0.26	3.76±0.32	4.58±0.53	4.79±0.23	4.75±0.14	5.02±0.12
	Infected	3.51±0.24	3.94±0.3	3.11±0.20	2.84**±0.16	3.18**±0.14	3.39**±0.17	4.19**±0.12
Albumin (g/dl)	Control	1.47±0.04	1.58±0.06	1.79±0.03	1.96±0.03	1.92±0.04	1.90±0.08	2.01±0.06
	Infected	1.57±0.05	1.51±0.17	1.37±0.17	1.55*±0.13	1.38**±0.13	1.55*±0.10	1.86±0.09
Globulins (g/dl)	Control	1.66±0.09	2.05±0.42	1.97±0.26	2.62±0.23	2.87±0.21	2.48±0.07	3.01±0.19
	Infected	1.63±0.07	2.43±0.40	1.74±0.29	1.16**±0.17	1.80*±0.24	1.83**±0.24	2.20**±0.11
Albumin/globulin ratio	Control	0.89±0.03	0.79±0.06	1.00±0.16	0.77±0.07	0.68±0.05	0.67±0.02	0.68±0.07
	Infected	0.96±0.02	0.75±0.22	0.93±0.24	1.50*±0.31	0.87±0.20	0.95±0.22	0.86±0.08
Iron (µg/dl)	Control	55.01±0.85	56.14±2.08	56.94±1.50	58.44±1.48	58.42±1.98	60.60±1.28	54.45±1.78
	Infected	54.70±0.49	44.81**±1.50	27.79**±1.21	25.31**±1.77	36.53**±2.08	47.43**±1.68	49.33*±1.15
Alanine aminotransferase (IU/l)	Control	20.0±0.7	21.20±1.31	26.20±0.96	25.20±0.66	24.20±1.1	22.20±1.39	23.00±1.09
	Infected	18.80±0.37	38.20**±1.42	58.00**±1.81	62.20**±1.82	61.20**±2.26	61.60**±1.53	49.20** ±1.24
Aspartate aminotransferase (IU/l)	Control	124.00±1.04	122.60±1.56	129.60±1.43	128.40±1.20	135.00±1.94	133.00±1.64	138.60±0.87
	Infected	121.80±0.86	160.20**±1.30	175.60**±2.87	178.20**±2.26	179.80**±2.51	176.20**±2.63	169.00**±2.58
Alkaline phosphatase (IU/l)	Control	168.80±1.06	167.60±0.92	168.60±0.29	171.20±1.51	170.00±1.81	171.80±1.20	176.00±1.50
	Infected	170.20±1.28	170.80±1.31	168.60±0.92	170.20±1.59	171.60±1.36	170.60±1.36	179.40±0.50
Uric acid (mg/dl)	Control	3.26±0.15	4.03±0.23	4.49±0.17	4.49±0.18	4.74±0.20	4.90±0.19	4.94±0.08
	Infected	3.51±0.15	6.50**±0.20	7.28**±0.19	7.22**±0.18	7.25**±0.16	5.16±0.24	5.57±0.26
Creatinine (mg/dl)	Control	0.95±0.01	0.95±0.03	1.00±0.03	1.04±0.07	1.03±0.05	1.05±0.05	1.11±0.05
	Infected	0.90±0.03	1.72**±0.03	1.90**±0.03	1.94**±0.03	1.62**±0.08	1.41**±0.04	1.41**±0.04

SE = Standard error. = * Significant at $P \leq 0.05$. ** = Highly significant at $P \leq 0.01$. Number of chicks = 5.

Compared to the normal control group, the leukogram of the infected group revealed significant leukocytosis and heterophilia started from the 2nd dpi till the 28th dpi which reached to the maximum at the 7th dpi. Significant lymphopenia started from 2nd dpi till the 14th dpi was detected in infected group. A significant increase in monocyte counts was observed at the 14th dpi. A marked decrease in eosinophil counts in infected group was noticed from the 2nd to the 7th dpi, while at the 21th dpi, the eosinophil count showed significant increase (Table 2).

Serum Biochemical Changes: The infected chicks showed marked drop in serum total proteins, albumin (A) and globulins (G) levels associated with an increase in the A/G ratio from the 7th dpi till the end of the experiment compared to control group (Table 3).

Compared to the control group, the infected chicks showed significant decrease in the level of serum iron from the 2nd dpi till the end of the experiment (Table 3).

The activities of ALT and AST showed highly significant increase with no significant changes in alkaline phosphatase activity in serum of the infected chicks from the 2nd dpi till the end of the experiment in comparison with the control group (Table 3).

Compared with the control group, the infected chicks showed a highly significant increase in serum creatinine started from the 2nd dpi till the end of the experiment, while serum uric acid showed a highly significant increase from the 2nd dpi till the 14th dpi (Table 3).

DISCUSSION

This study was carried out to investigate the effect of experimental infection of one-day-old chicks with locally isolated *Salmonella* Gallinarum strain on hematological and biochemical constituents.

The recorded clinical signs following *S.* Gallinarum infection were dullness, ruffled feathers, droppings, huddle together, white pasty diarrhea, loss of appetite, decrease in feed intake and depression. These clinical signs began to appear at the beginning of the 2nd dpi suggesting that the incubation period of the disease was about 24 hours. These clinical signs were also reported by many investigators [6, 9, 29].

Regarding to erythrogram of the experimentally infected group with S. Gallinarum, significant decrease was detected in RBCs count and Hb concentration with macrocytic hypochromic anaemia that may be attributed to hemolysis of erythrocytes and release of reticulocytes to the peripheral circulation. In addition, degenerative changes in the liver and destruction of the intestinal mucosa lead to deficiency of vitamin B_{12} due to interference with its absorption in intestine and its storage in liver [19], so macrocytic anaemia developed. These results agreed with Assoku *et al*. [13] and Hegab [30] while these results disagreed with Christensen *et al*. [31] and Mdegela *et al*. [32] who reported microcytic hypochromic anaemia during acute *S.* Galinarum infection.

Leukogram revealed significant leukocytosis and heterophilia all over the experimental periods with significant lymphopenia till the 14th dpi in the infected group compared with the control one. This lymphopenia may be due to stress of infection with *S.* Gallinarum which stimulate the adrenal gland to secrete corticosteroid hormones, causing destruction of lymphocytes [33]. The endogenous glucocorticoids causes heterophilia associated with lymphopenia, eosinopenia and monocytopenia or monocytosis, but the continuous endogenous release of glucocorticoids results in sustained changes in leukocyte number, particularly eosinophilia and lymphopenia [34]. An increase in the value of heterophils in the infected chicks may be related to that heterophils is the most cell of defense in the body and it is the first line of defense which attack and engulf the microorganisms as a normal response to bacterial *Salmonella* infection [19]. These results were in harmony with Msoffe *et al*. [35] and Gheith [36], while different result was recorded by Freitas Neto *et al*. [6] who reported leukopenia followed by leukocytosis.

Regarding to the results of total serum proteins, albumin and globulins in infected group, significant hypoproteinaemia, with hypoalbuminaemia and hypoglobulinaemia were demonstrated started from the 7th dpi till the end of the experiment. Hypoproteinaemia may be due to decrease in feed intake and malabsorption through the intestine due to enteritis. The decrease in albumin concentrations may be attributed to the view that hypoalbuminaemia is an important parameter for the liver damage as the liver is the only organ where albumin is synthesized as well as alpha and beta globulins [37]. During sepsis, acute phase proteins are preferentially produced in the liver and thus, albumin synthesis is inhibited and due to its small size molecule, albumin is selectively lost in renal and intestinal diseases such as acute fowl typhoid [38]. Also the decrease in globulins might be due to the destructive processes in infected chicks [38]. These recorded results disagreed with result of Hegab [30] who reported a significant increase in the levels total proteins and globulins.

The present work showed significant decrease in serum iron concentration all over the experimental period. This result added more support to those previously with the results of Kokosharov and Todorova [39] who recorded decrease in serum iron concentration in chicks experimentally infected with *S.* Gallinarum, also it causes disturbance in iron regulation, which cause shortage of iron in the body and disturbance of iron metabolism.

Assessment of serum biochemistry revealed a significant increase in serum liver enzymes (AST and ALT) activities from the 2nd dpi till the end of the experimental periods in infected group. This increase could be suggestive of hepatic affection as AST and ALT are good indicators of hepatocellular damage [40]. Regarding to ALP, there were no significant changes. These results agreed with the results of Gheith [36], while disagreed with Hegab [30] who reported a significant increase in the level of alkaline phosphatase.

The present work showed significant increase in serum uric acid and creatinine levels that may be attributed to kidney damage caused by *Salmonella* infection [40]. This result agreed partially with the results of Hegab [30], Gheith [36] and Ahmed *et al*. [40] who reported a significant increase in serum uric acid and creatinine in experimentally infected chicks with *Salmonella.* The level of creatinine and uric acid is known to reflect the state of glomerular filtration rate and kidney function [41].

CONCLUSION

Experimental *S.* Gallinarum infection in chicks induced acute anaemia, leukocytosis, heterophilia, lymphopenia and alteration in liver and kidney functions. The demonstrated acute anaemia is considered as one of the diagnostic tools for acute fowl typhoid disease.

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