Clinicopathological Studies On Naturally Infected Chickens With Avian Influenza

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ABSTRACT

Forty, 6 week old Hubbard breed chickens (twenty were clinically healthy and the other twenty were naturally infected with avian influenza H5N1) were used for hematological and biochemical studies in this work . Blood samples were collected by heart puncture on the 3rd day post appearance of clinical signs. Specimens were collected from the liver, kidneys, spleen, heart and intestine .The specimens were fixed in 10% neutral buffered formalin.Five micron thick paraffin sections were prepared and stained with hematoxylin and eosin for histopathological examination.

Normocytic normochromic anemia, leukopenia, lymphopenia, heteropenia and monocytopenia were encountered in the diseased group. The lymphocyte transofmration rate and phagocytic % were decreased. The serum aspartate aminotranferase (AST), alanine aminotranferase (ALT), globulin, creatinine and uric acid were highly significantly increased together with a significant increase in the serum phosphorus in the infected chickens. Moreover hypocalcemia and hypoprotienemia with a highly significant decrease in the serum albumin, albumin/globulin ratio, cholesterol, triglyceride, high density lipoprotein, low density lipoprotein and very low density lipoprotein when compared with the normal control group.Microscopically, the hepatic parenchyma revealed hyperemia, edema and hemorrhages. Moreover, degenerative or necrotic parenchymal cells associated with perivascular and intestitital lymphocytic aggregations were common.Focal or diffuse hemorrhages associated with focal coagulative necrosis of renal tubules could be seen.The spleen showed focal necrosis of white pulps accompanied lymphoid depletion and proliferation of reticular cells in the red pulps with heterophilic infiltration and hemorrhages in the subscapsular tissues were common. The Myocardium showed mycarditis characterized by necrosis associated with lymphoid infiltration scattered among necrotic muscles, beside hemorrhages, edema and lymphocytic infiltration on the pericardium. Intramuscular edema and hyperemia of blood vessels were outstanding. The intestinal lumina contained blood clots mixed with mucus beside goblet cells metaplasia in villus epithelium and glands with edema and hemorrhages in the intestinal mucosa.

INTRODUCTION

Avian influenza (AI) viruses are members of family Orthomyxoviridae. Avian influenza disease is not only one of the most serious problems responsible for high economic losses in the poultry industry *(1)* but is also zoonotic with a lethal effect *(2)*. The first record for avian influenza was in 1878 by Perroncito in Italy and the disease was confused with acute septic form of fowl cholera until 1880 when Rivalto and Delprato differentiated between them, depending on the clinical and pathological features. The virus was classified as an avian influenza virus in 1955 *(3)*. The morbidity and mortality rates of the highly pathogenic avian influenza viruses may approach 100% *(4-6)*.

The clinical signs of avian influenza are sudden onset of high mortality, respiratory signs, rales, excessive lacrimation, sinusitis, edema of the head and face, subcutaneous hemorrhage with cyanosis of the skin, particularly of the head and wattles besides diarrhoea *(3)*. The gross lesions included congestion and hemorrhages on the skin, liver, spleen , kidneys, heart and lungs *(7)*. The avian influenza virus has a damage effect on both the polymorphonuclear and mononuclear phagocytes besides T and B lymphocytes through a direct effect rather than by

replication leading to depletion of the cells from essential components *(8)*.

The aim of the present work is to evaluate the hemogram, blood chemistry, cellular immunity and associated lesions in chickens naturally infected with avian influenza.

MATERIAL AND METHODS

Six weeks old 40 chickens (twenty were clinically healthy (gp.1) and the rest (gp.2) were naturally diseased with avian influenza H5N1) were obtained from Al-Magry company and used in this work .The disease was diagnosed by the National Lab. for Veterinary Quality Control for Poultry Production as H5N1 (Authorites).Three blood samples were collected at the same time by heart puncture from gps. (1&2). The first blood sample (0.5ml) was collected from each chicken on EDTA and used for the estimation of the erythrocytic and total leukocytic counts *(9)* packed cell volume *(10)* and hemoglobulin using the cyanmethemoglobin colorimeteric method *(11)*. The differential and absolute leukocytic counts was carried out *(10)*. The second blood sample (2ml) was collected in a sterile plastic centrifuge tube containing heparin (50 IU/m) to be used for the estimation of lymphocyte transformation rate(LTR) *(12)* and phagocytic percentage *(13)*. The third blood sample (5 ml)was collected in a centrifuge tube to separate the serum for the determination of serum total protein *(14)*, albumin *(15)*, globulin *(16)* ,AST and ALT *(17)*, triglyceride *(18)*, cholesterol *(19)*, high–density lipoprotein cholesterol (HDL-C) *(20)*, low-density lipoprotein cholesterol (LDL-C) and very low – density lipoprotein cholesterol (VLDL-C) *(21)*, creatinine *(22)*, uric acid *(23)*, calcium *(24)*, and inorganic phosphorus *(25)*.

The clinical signs were recorded.The birds was necropsied and specimens were collected from the liver, kidneys, spleen, heart and intestine.The specimens were fixed in 10% neutral buffered formalin. Five micron thick paraffin sections were prepared and stained by hematoxylin and eosin and examined microscopically *(26)*.

Results of tested parameters were statistically analyzed *(27)* using the MSTAT – C computer program.

RESULTS

The diseased chickens showed a sudden onset of high mortality,besides depression, decreased food consumption and diarrhea .Moreover respiratory manifestation, cyanosis and edema of the comb and wattles (Fig.1) and the shank of the leg contained streaks of hemorrhage (Fig.2) .

Table (1) shows a highly significant decrease in the number of RBCs , hemoglobin and packed cell volume with development of normacytic normochromic anemia in the diseased group (gp.2).

Table (2) shows leukopenia, lymphopenia, heteropenia, monocytopenia besides a highly significant decrease in the lymphocyte transformation rate and phogocytic percentage.

Gp.(2) showed a highly significant increase in both the AST and ALT. Hypoproteinemia, hypoalbuminemia and a decreased albumin/ globulin ratio were encountered while a high significant increase was recorded in the globulin (Table 3). A highly significant decrease was found in the serum cholesterol, triglycerdes, high density lipoprotein, low density lipoprotein and very low density lipoprotein (Table 4). Table (5) shows a highly significant increase in both the serum creatinine and uric acid. The serum Ca showed a significant decrease while P was significantly increased compared with the normal control .

Gp.(1) showed no lesions. The liver (gp.2) was enlarged and showed petechial haemorrhages. Microscopically, the hepatic parenchyma revealed hyperemia, edema and hemorrhages (Fig.3). Moreover, degenerative and necrotic changes were associated with perivascular and interstitital lymphocytic aggregations (Fig.4). Macroscopically, the renal lobules were slightly enlarged and

congested . Microscopically, focal and diffuse hemorrhages were associated with focal coagulative necrosis of the renal tubules (Fig. 5). Macroscopically, the spleen was slightly reduced in size with areas of petechial hemorrhages. Microscopically, focal necrosis of the white pulp was accompanied by lymphoid depletion and proliferation of the reticular cells in the red pulp with heterophilic infiltration and hemorrhages in the subscapsular tissues (Fig.6). Macroscopically , the pericardium was hemorrhagic. Microscopically, the myocardium showed mycarditis characterized by necrosis and lymphocytic infiltration among the necrotic muscles, besides hemorrhages, edema and lymphocytic infiltration in the pericardium (Fig.8). Intramuscular edema and hyperemia (Fig. 2.B). Macroscopically, the intestinal mucosa was congested and the intestinal contents were watery. Microscopically,the intestinal lumina contained blood clots mixed with mucus besides numerous goblet cells covering the villi and lining the glands with edema and hemorrhages in the intestinal mucosa (Fig. 9).

Table 1. Hemogram of the chickens (mean values ± SE).

Parameters / Groups	RBCs (x 10^6/µl)	PCV %	Hb gm/dl	MCV Fl	MCHC %
(normal control) 1	3.21 +0.02	29.30 +0.20	10.33 +0.09	91.28 +0.43	35.20 +0.22
(diseased group) 2	2.49** +0.01	22.70** +0.27	8.03** +0.11	91.11 +0.51	35.23 +0.17
% difference	-22.43	-22.53	-22.27	-0.13	+0.31

** Highly significant at P≤0.01

Table 2. Leukogram, lymphocyte transformation rate and phagocytic % of the chickens (mean values ± SE).

Parameters / Groups	Total and absolute differential leukocytic count (x10^3/µl)				LTR	Phogocytic %
	TLC	Heterophil	Lymphocyte	Monocyte		
(normal control) 1	23.57 +0.05	6.03 +0.03	15.12 +0.02	1.92 +0.03	1.67 +0.01	80.90 +0.44
(diseased group) 2	18.99** +0.16	3.94** +0.06	13.04** +0.09	1.56** +0.03	1.24** +0.01	71.15** +0.49
% difference	-19.43	-34.66	-13.76	-18.75	-25.75	-12.05

**Highly significant at P≤0. 01

Table 3. Some liver function tests in the serum of the chickens (mean values ± SE).

Parameters / Groups	AST (U/L)	ALT (U/L)	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	A/G
(normal control) 1	57.25 +0.48	8.30 +0.18	4.07 +0.02	2.18 +0.02	1.89 +0.02	1.16 +0.01
(diseased group) 2	69.40** +0.91	11.95** +0.32	3.71* +0.14	1.51** +0.03	2.20** +0.02	0.69** +0.01
% difference	+21.22	+43.48	-8.60	-30.73	+16.40	-40.51

*Significant at P≤0.05 ** Highly significant at P≤0.01

Table 4. Lipogram of the chickens (mean values ± SE).

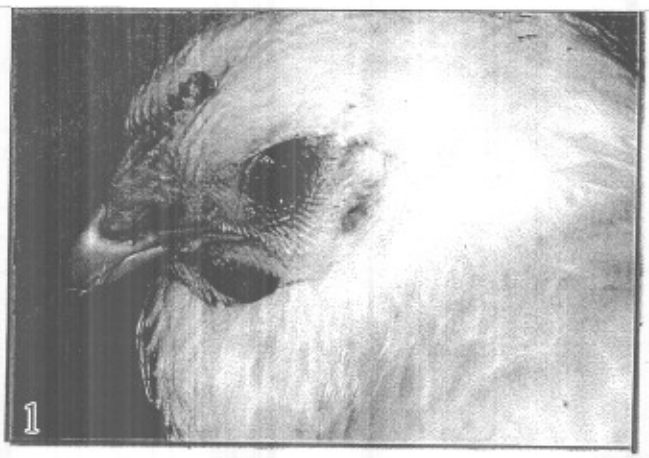
Parameters / Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
(normal control) 1	131.01 +0.42	89.88 +0.49	74.60 +0.33	38.25 +0.17	17.98 +0.10
(diseased group) 2	118.10** +0.52	78.00** +0.39	68.00** +0.37	34.52** +0.14	15.60** +0.08
% difference	-9.85	-13.22	-8.85	-9.75	-13.24

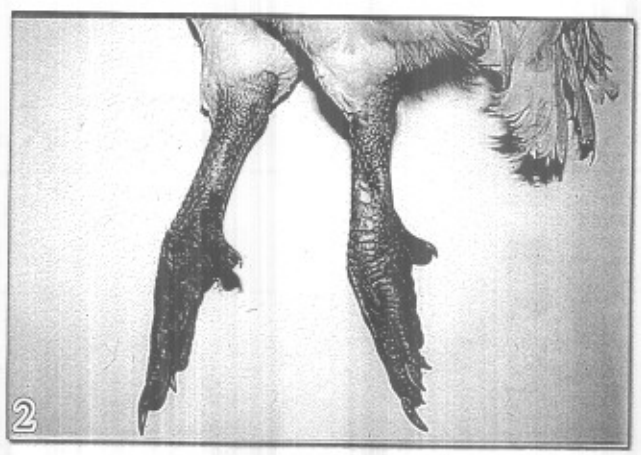
** Highly significant at P≤0. 01

Table 5. Some biochemical tests in the serum of the chickens (mean values ± SE).

Parameters / Groups	Creatinine (mg/dl)	Uric acid (mg/dl)	Ca (mg/dl)	P (mg/dl)
(normal control) 1	0.86 +0.02	4.78 +0.12	11.74 +0.21	5.33 +0.13
(diseased group) 2	1.44** +0.04	6.96** +0.11	10.78* +0.30	5.93* +0.20
% difference	+67.44	+45.61	-8.18	+11.25

*Significant at P≤0.05 **Highly significant at P≤0. 01

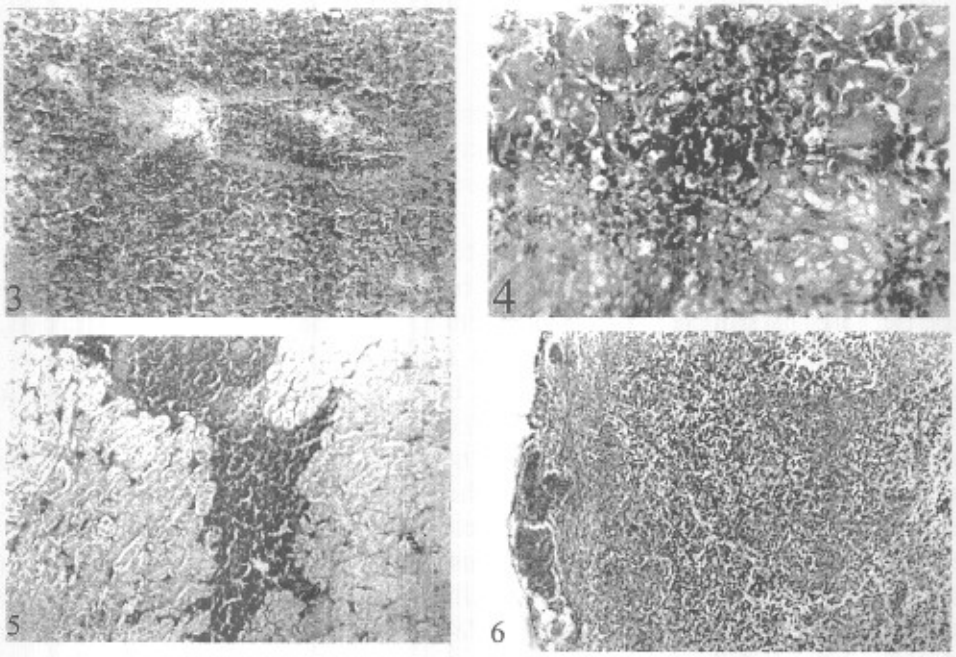




Figs. (1&2), gp. (2).

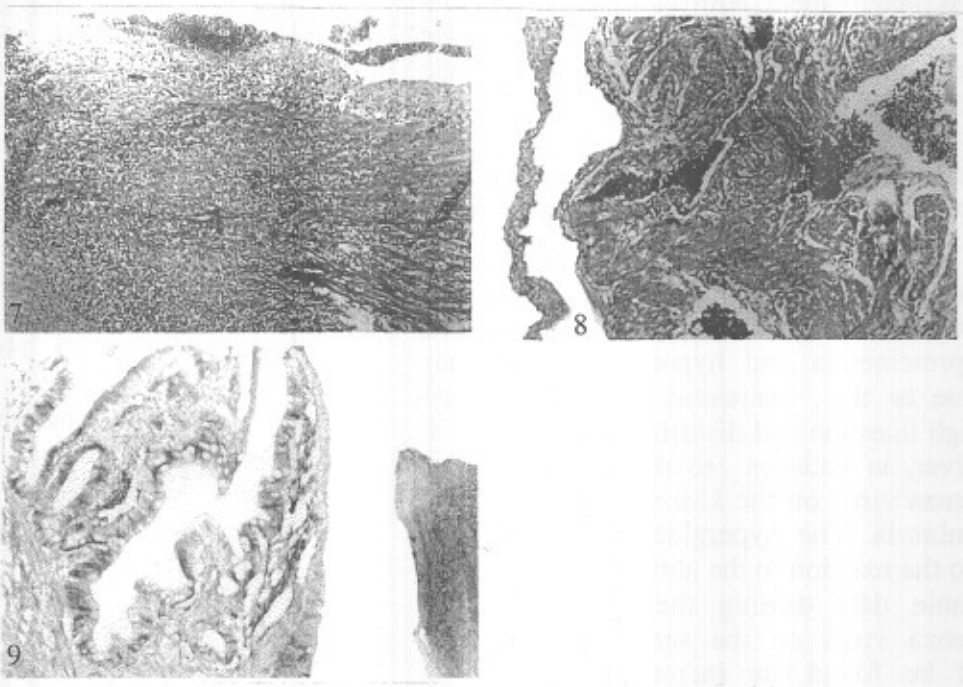
1. Broiler chicken showed cyanosis and edema of the comb and wattles (Fig.1) and the shank of the leg contained streaks of hemorrhage (Fig.2) .

2. Broiler chicken showed hemorrhage in the shank of the legs.



Figs. (3-6), gp.(2).

3. Liver showing hyperemia, hemorrhage and edema besides leukocytic aggregates (H & E. , 150).
4. Liver showing necrotic areas infiltrated with lymphocytes (H & E. , x 300).
5. Kidney showing hemorrhages and focal coagulative necrosis of the renal tissue (H & E. , 150).
6. Spleen showing subcapsular haemorrhages and lymphocytic necrosis (H & E., x 150).



Figs. (7-9),gp.(2).

7. Mycoarditis and epicardial hemoarrhages (H & E., 150)
8. Myocardium showing hyperemia and intermuscular edema (H & E ., x 150).
9. Intestine showing goblet cell metaplasia of the villi epithelium and exudate inside the intestinal lumen (H & E., x 150).

DISCUSSION

The clinical signs, observed in the avian influenza diseased chickens, were previously mentioned by several investigators *(3 & 7)*. The picture of the erythron mass, in the diseased group, was normocytic normochromic anemia. This anemia may be due to the hemorrhages present in the different organs or decreased red cell production as a result of the kidney lesions.The leukopenia is due to lymphopenia, heteropenia and monocytopenia. Such a decrease in the lymphocytes and phagocytic cells (heterophil and monocyte) could be due to the direct damage effect of the avian influenza virus, rather than its replication,depleting the cells from essential components *(8)*. Moreover, *(28)* reported a depletion of the lymphocytes from the splenic white pulp in 2 day old chicks infected with avian influenza (H_5N_3) which gets along with the lymphopenia.

The lymphocyte transformation rate and phagocytic % was decreased in gp.(2). This is inagreement with previously obtained results *(8)* who reported that the phagocytes are less chemotactic and less able to ingest and kill bacteria and lymphocytes also seem to have impaired function in the avian influenza.The lymphoid depletion of the spleen confirms our results.

The proteinogram of group (2) revealed hypoproteinemia, hypoalbuminemia and decreased albumin / globulin ratio. The hypoproteinemia and hypoalbuminemia may be due to the decreased feed intake, loss through intestine and disturbed metabolism of the liver, in addition to the effect of avian influenza virus on the kidneys which leads to albuminuria. The hyperglobulinemia may be due to the reaction to the antigenic stimuli . No available data dealing the effect of avian influenza virus on the serum proteinogram could be found.The increased activities of aminotrasnferases (AST and ALT) were associated with hepatocellular damage ***(29).*** The present study showed a highly significant increase in the serum AST and ALT activities in the diseased group. This may be due to the degenerative changes, induced by avian influenza virus in the liver and heart. The previous results could be correlated with hyperemia, edema and hemorrhages of the hepatic parenchyma. Moreover, degenerative and necrotic parenchymal cells were associated with perivascular and interstitial lymphocytic aggregations. Myocarditis, characterized by necrosis, lymphocytic infiltration and hemorrhages were seen.The pericardium showed edema and lymphocytic infiltration.Nearly similar findings were reported in the liver and heart ***(3).*** A highly significant decrease in the levels of the cholesterol, triglycerides, high density lipoprotein, low density lipoprotein and very low density lipoprotein was seen. Such decrease may be attributed to the decreased intestinal absorption ,due to enteritis, and the decreased hepatogenic lipogenic activity due to hepatic damage ***(30).*** The microscopical picture of both the liver and intestine (gp.2) gets along with the current results .

The present work showed that the avian influenza virus damaged the renal tissue as clarified by both the clinico–and histopathological means. This renal damage was reflected by an increased serum uric acid, creatinine and phosphorus with hypocalcemia. Hyperurecemia, in birds, occurs with starvation, gout, massive tissue destruction and renal diseases *(29)*. Some investigators think that the serum creatinine may become elevated in birds with renal diseases, but less reliably than the uric acid ***(31)***. Hyperphosphatemia may be due to a decrease in the calcium level and an increase of the parathormone hormone as a result of hypocalcemia .The latter may be attributed to a reduced renal calcium reabsorption, decreased calcium absorption from the intestine, increased excretion and /or hypoalbuminemia *(29)*. Such biochemical changes, in the present work, are the outcome of nephropathy which is manifested by focal and diffuse hemorrhages associated with focal coagulative necrosis of the renal tubules. Nearly similar findings was reported *(3)*. It could be concluded that the avian influenza showed a deleterious effect on the blood

forming tissues ,parenchymatous organs and vasculature.

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الملخص العربى

دراسات باثولوجية إكلينيكية على تأثير مرض أنفلونزا الطيور فى الدجاج

نصر عبدالوهاب محمد نصر الدين

قسم الباثولوجيا الإكلينيكية – كلية الطب البيطرى – جامعة الزقازيق

أجريت هذه الدراسة على عدد ٤٠ دجاجة هبر عمر ٦ أسابيع (عشرون دجاجة غير مصابة والعشرون الأخيرة مصابة بمرض انفلونزا الطيور وقد تم أخذ عينات الدم من القلب فى اليوم الثالث من ظهور الأعراض كما تم أخذ عينات من الكبد والكلى والطحال والقلب والأمعاء وقد أظهرت النتائج أن المجموعة المريضة كانت تعانى من أنيميا الدم ذات الخلايا الطبيعية الشكل والهيموجلوبين مع نقص فى العدد الكلى لخلايا الدم البيضاء والخلايا الليمفاوية والخلايا متعادلة الصبغة والخلايا وحيدة النواه وأيضا وجد نقص فى معدل تحول الخلايا الليمفاوية ونسبة الخلايا البلعمية كما أظهرت النتائج زيادة فى أنزيمات الآلانين أمينوترانسفيريز والأسبرتيت أمينوترانسفيريز والجلوبيولين والكرياتنين وحمض البوليك والفسفور غير العضوى مع نقص البروتين الكلى والزلال ونسبة الزلال إلى الجلوبيولين والكوليستيرول الكلى والجلسيريدات الثلاثية ، البروتينات الدهنية عالية الكثافة والمنخفضة الكثافة وشديدة انخفاض الكثافة والكالسيوم مقارنة بالمجموعة الضابطة.