Hematological biochemical immunological and pathological studies on pasteurellosis in chicken

By
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SUMMARY

Forty five 4 weeks old Balady chicken were divided into three groups. Group (gp) 1 15 birds used as control group,gp2 twenty birds and gp3 ten birds. Group2 and 3 were intramuscularly injected with 0.2 ml/birds of 8 hrs broth culture of Pasteurella multocida (p.m) containing 3x10^8 viable organisms of p.m / ml. Gp 3 was treated with gentamycin intramuscularly three days post infection at a dose of 4mg/kg boy weight for 5 successive days. Clinical signs, mortality rate, hematological changes ,serum biochemical changes , post mortem lesion and histopathological examination were studied. Blood samples were collected from the wing vein, 3 , 10 and17 days post infection (PI) from gp 1 & 2 , and1 and 2 week post treatment( PT) from gp3. Blood samples were divided into 2 parts .The first part was collected on EDTA for detection of RBCs count ,HB concentration, PCV, WBCs and differential leukocytic count. The second part was collected into plain centrifuge tube for serum separation and determination of total protein, electrophoretic pattern of serum protein , AST,ALT and GGT, serum calcium, phosphorus and uric acid. Tissue specimens were taken from liver , kidney and spleen 3, 10 and 17 day pl. from chicken of gp 1 and 2, one and two weeks PT from gp 3 . Results of the changes in hemogram revealed significant decreased of RBCs count HB and PCV values in gp2.Total leukocytic count was highly significant decreased 10 and 17 days PI in gp2. Heteropenia was observed 3and 10 days PI in gp2.Monocytes was significantly increased in gp2 three days pi. Parameters of hemogram in gp 3 were non significantly changed from that of the control gp . Results of the changes in serum biochemical parameter revealed significant increased in AST, uric acid and non significant increase in ALT and GGT in gp 2 three and ten days PI. Determination of total protein and its fractions reveled significant decreased in albumin with decreased in A/G ratio in gp 2 three days PI .Protein fractions revealed increased values in beta and gamma globulin in gp 2 three and ten days PI. Non significant increased in gamma globulin were observed 3 & 10 days PI in gp 2. The histopathological examination of the liver showed degenerative changes of the hepatocytes , congestion of blood vessels with hemolytic RBCs in the lumen and focal heterophile infiltration. Degenerative changes of the hepato-
cytes, congestion of blood vessels, focal heterophilic infiltration and coagulative necrosis. The spleen showed lymphocytic depletion 3 & 10 days post infection. Kidney showed focal interstitial nephritis with degenerative changes in the renal tubular epithelial cells.

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INTRODUCTION

Bacterial disease are one of the most impotent problem facing poultry industry in Egypt. Fowl cholera is a highly contagious disease infected many species of domesticated and wild birds (Botzler, 1991). Pasteurella multocida is the causative agent (Adlam and Rutter, 1989). In developing countries P. multocida was isolated from 25.9% of apparently healthy and 6.2% of chicken from free range family poultry farm and slaughter slabs at market (Mbuthia et al., 2008). Wild birds may be a source of infection to commercial poultry. Carrier birds play a major role in the transmission of disease. Infection may range from per acute, acute to chronic infection (Christensen and Bisgaard, 2000). The acute course of the disease is characterized by high morbidity and mortality rates with septicemia lesions. The chronic form results in localization of the infection in wattles and respiratory passages and to a lesser extent in the ovaries and joints (Rhodes and Rimler, 1984). Heddleston and Reber (1975) mentioned that the endotoxins of P. multocida was toxic for chicken. Diallo and Frost (2000) found that P. multocida extract has hemolytic effect on chicken RBCs. The vascular disturbance with degenerative and necrotic changes which occurred in the parenchymatous organs, could be due to P. multocida toxin (Taha et al., 1986). Sarkozy et al. (2002) succeeded in inducing experimental fowl cholera in 10 weeks old broiler chicken. Woo and Kim (2006) isolated P. multocida from two outbreaks of fowl cholera in Korea. Chicken experimentally infected with P. multocida showed an increase in AST, ALT, uric acid, creatinine and phosphorous with decrease in calcium and albumin (Amany, 1997). Gentamycin an aminoglycoside antibiotic is wildly used for treatment of various infectious diseases. In poultry medicine, genta-
mycin is wildly used for treatment of fowl cholera and other bacterial infection. Booth (1988) and Karaivanov (1983) reported that P. multocida was sensitive to gentamycin.

The present work was designed to study the hematological, serum biochemical and pathological changes in chicken infected by P. multocida pre and post treatment.

MATERIAL AND METHODS

Birds:
Forty five 4 weeks old Balady chickens proved to be free from pathogenic bacteria and parasitic infestation were used in this study. Chicken were divided into three groups. Group (gp) 1 ten birds used as control group, gp2 twenty birds and gp3 ten birds. Group 2 and 3 were intramuscular infected with 0.2 ml/ birds of 8 hrs broth culture of P multocida containing 3x10^8 viable organisms of / ml (Amany, 1997). Gp 3 was treated with gentamycin intramuscularly three days post infection at a dose of 4mg/kg body wt. for 5 successive days. Clinical signs, number of death, hematological changes, serum biochemical changes, post mortem lesion and histopathological examination were recorded. Pasteurella multocida strain: Pasteurella multocida type (A) was obtained from Vet. Serum and Vaccine Research Institute, Abbasia, Cairo. This strain had been passaged in rat to maintain its virulence and reisolated from rat on blood agar plates.

Drugs: Gentamycin was obtained from Amoun Pharmaceutical CO. It was given intramuscularly at a dose 4mg/kg body wt for 5 successive days according to Sarkozy et al. (2002).

Diagnostic kits: Commercial diagnostic kits were purchased from Spinreact, Diamond, Egypt and Biodiagnostic for the determination of hemoglobin, total protein, aspartate aminotranseferase (AST), alanine aminotranseferase (ALT), gamma glutamyl transferase (GGT), calcium (ca) ,phosphorus (ph) and Uric acid.

Samples:
Blood samples were collected from the wing vein, 3, 10 and 17 days post infection (PI) from gp 1 & 2, and 1 and 2 week post treatment pt from gp3. The blood samples were divided into 2 parts. The first part was collected on EDTA for detection of RBCs count, Hb concentration, PCV ,WBCs and differential leukocytes count according to Fildman et al. (2000). The second part was collected into plain centrifuge tube for serum separation and determination of total protein according to Henry et al. (1974), electrophoretic pattern
of serum protein according to Nils (1983), aspartate aminotransferase, alanine aminotransferase activities according to Ritman and Frankel (1957) gamma glutamyl transferase activity (GGT) according to Bernard (1991), serum calcium according to Sarkar and Chanhwan (1967), serum phosphorus according to Goodwin (1970) and uric acid level according to Young (2001).

RESULTS

Clinical signs:
Chicken infected with P. multocida Pm showed anorexia, depression, ruffled feather, drowsiness, diarrhea and loss of body weight. Mortality rate among chicken was 25% at 1 and 2 days PI.

Results of clinical pathology:--

a- Hemogram:
Results of the changes in hemogram in chicken of the experimental groups are shown in Table (1). It revealed that values of RBCs count, HB and PCV were significantly decreased in gp 2 during the experimental period. Total leukocytic count was highly significant decreased 10 and 17 days PI in gp 2. Heteropenia was observed 3 and 10 days PI in gp 2. Monocytes was significantly increased in gp 2 three days post infection. Parameters of hemogram in gp 3 were non significantly changed from that of the control gp.

b- Serum biochemistry:
Results of the changes in serum biochemical parameter in chicken of the experimental groups are shown in Table (2). Examination of serum enzyme revealed significant increased in AST and non significant increase in ALT and GGT in gp 2 three and ten days PI. Uric acid showed increased values in gp 2 during the experimental period. Serum calcium was decreased significantly 3 and 10 days PI in gp 2 while phosphorous was increased significantly 3 days PI. Determination of total protein and its fractions revealed significant decreased in albumin with decreased in A/G ratio in gp 2 three days PI. The 4 serum protein electropho-
retic fractions revealed increased values in beta and gamma globulin in gp 2 three and ten days PI. Non significant increased in gamma globulin were observed 3 and 10 days PI in gp 2.

Pathological examination:
The gross lesion:-
The gross lesion in infected chicken showed congestion of the muscles in gp 2, 3 days PI Fig (1). The liver of some affected chicken in gp 2 was congested and showed necrotic foci Fig (2). General hyperemia was most evident in the blood vessels' of the abdominal viscera.

The histopathological examination:-
Liver: The histopathological examination of the liver in gp 2 three days PI showed diffuse degenerative changes of the hepatocytes, congestion of blood vessels with hemolytic RBCs in the lumen (Fig., 3) and focal heterophilic infiltration (Fig., 4). Coagulative necrosis in liver of gp 2 was observed 17 days PI (Fig. 5)

Spleen: The spleen of chicken in gp 2 showed lymphocytic depletion 3and10 days PI (Fig. 6).

Kidney: Kidney showed focal interstitial nephritis with, degenerative changes in the renal tubular epithelial cells with focal heterophilic infiltration (Fig. 7).

DESCRIPTION OF FIGURES
Fig. 1: Chicken of gp 2 three days PI showing congestion of the muscles.
Fig. 2: Liver of chicken of gp 2 at 3 days PI showing congestion and presence of necrotic foci.
Fig. 3: Liver of chicken in gp 2 at 3 days PI showing congestion of blood vessels with hemolytic RBCs in the lumen (H&E-x200).
Fig. 4: Liver of chicken in gp 2 showing focal leucocytic infiltration (H&E-x200).
Fig. 5: Liver of chicken in gp 2 at 17 days PI showing coagulative necrosis.(H&E-200).
Fig. 6: Spleen of chicken in gp 2 showing lymphocytic depletion (H&E-x200)
Fig. 7 : Kidney of chicken in gp 2 showing degenerative changes in the renal tubular epithelial cells with focal heterocyst infiltration (H&E-x200).
Fig. 8 : Spleen of chicken in gp 2 at17 days PI showing lymphocytic depletion (H&E-x 200).
Fig. 9: Spleen of chicken in gp 2 at17 days PI showing angiopathy (H&E-x200).
Fig.10: Electrophoretic pattern of serum proteins and analysis showing different protein fractions.
Table (1) : Mean values and standard error of hemogram in different experimental groups of chicken.

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>RBCs x 10^6 ul</th>
<th>Hb gm/dl</th>
<th>Pcv %</th>
<th>WBCs x10^3 ul</th>
<th>Differential leucocytic count %</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lymphocyt</td>
</tr>
<tr>
<td>3 days</td>
<td>1</td>
<td>2.06 ± 0.13</td>
<td>12.08 ± 0.4</td>
<td>29.11±0.9</td>
<td>30 ± 3.13</td>
<td>65 ± 2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.53±0.18 *</td>
<td>9.24±0.7 *</td>
<td>25±1 *</td>
<td>20.2 ± 4</td>
<td>61 ±6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.11±0.1</td>
<td>11.86±0.4</td>
<td>29.11±0.9</td>
<td>30.25 ±1.7</td>
<td>65.2 ± 2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.45±0.09 **</td>
<td>8.27±0.5 *</td>
<td>23.5±1 *</td>
<td>18±2 **</td>
<td>70 ±.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.87±0.06</td>
<td>9.42±0.9</td>
<td>26.25±1</td>
<td>25.3±0.8</td>
<td>60 ± 2</td>
</tr>
<tr>
<td>10 days</td>
<td>1</td>
<td>2.24±0.1</td>
<td>11.9±0.6</td>
<td>29.6±1</td>
<td>30.2 ±1.7</td>
<td>65 ±2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.7±0.1 *</td>
<td>9.65±0.5 **</td>
<td>25±0.8 *</td>
<td>20 ±2 **</td>
<td>70 ±3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.97±0.08</td>
<td>10.64±0.3</td>
<td>29.4±1</td>
<td>29.57±0.4</td>
<td>62±.3</td>
</tr>
</tbody>
</table>

Group 1 Normal control
Group 2 Infected
Group 3 Treated
* Significantly different from normal control group, P < 0.05
** Highly significantly different from normal control group, P < 0.001
Table (2): Mean values and standard error of some serum biochemical parameters in different experimental groups of chicken.

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>AST U/L</th>
<th>ALT U/L</th>
<th>GGT U/L</th>
<th>Uric Acid mg/dl</th>
<th>Calcium mg/dl</th>
<th>Phosphorus mg/dl</th>
<th>Total protein gm/dl</th>
<th>Protein Fractions gm/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Alb.</td>
</tr>
<tr>
<td>3 days</td>
<td>1</td>
<td>122.56 ± 10</td>
<td>10.03 ± 2</td>
<td>14.47 ± 0.9</td>
<td>5.82 ± 0.5</td>
<td>8.1 ± 1</td>
<td>3.87 ± 0.9</td>
<td>3.9 ± 0.1</td>
<td>2.34 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>171.8 ± 5*</td>
<td>11.44 ± 1</td>
<td>16.24 ± 0.9</td>
<td>10.94 ± 1*</td>
<td>3.6 ± 1*</td>
<td>5.9 ± 0.2*</td>
<td>3.72 ± 0.2</td>
<td>1.79 ± 0.1*</td>
</tr>
<tr>
<td>10 days</td>
<td>1</td>
<td>122.6 ± 10</td>
<td>10.08 ± 2</td>
<td>14.47 ± 0.9</td>
<td>5.82 ± 0.5</td>
<td>8.1 ± 1</td>
<td>3.8 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>2.34 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>169.8 ± 4*</td>
<td>12.56 ± 0.5</td>
<td>19.66 ± 1.6</td>
<td>9.71 ± 1</td>
<td>2.29 ± 0.4*</td>
<td>4.5 ± 0.5</td>
<td>4.03 ± 0.2</td>
<td>2.4 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>132.82 ± 17</td>
<td>11.9 ± 1</td>
<td>17.6 ± 2</td>
<td>7.99 ± 2</td>
<td>7.72 ± 2</td>
<td>4.1 ± 0.1</td>
<td>3.87 ± 0.5</td>
<td>2.62 ± 0.15</td>
</tr>
<tr>
<td>17 days</td>
<td>1</td>
<td>122 ± 3</td>
<td>10.08 ± 2</td>
<td>14.47 ± 0.9</td>
<td>5.82 ± 0.5</td>
<td>8.37 ± 1</td>
<td>3.8 ± 0.3</td>
<td>3.9 ± 0.1</td>
<td>2.34 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>128 ± 1</td>
<td>11.04 ± 0.9</td>
<td>18.6 ± 2</td>
<td>8.17 ± 0.8*</td>
<td>5.15 ± 1</td>
<td>3.1 ± 0.1</td>
<td>3.76 ± 0.1</td>
<td>2.024 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>117 ± 7</td>
<td>10.36 ± 1</td>
<td>14.67 ± 1.6</td>
<td>5.27 ± 0.1</td>
<td>8.25 ± 2</td>
<td>4 ± 0.8</td>
<td>3.28 ± 0.3</td>
<td>1.725 ± 0.17</td>
</tr>
</tbody>
</table>

Group 1 Normal control
Group 2 Infected
Group 3 Treated
* Significantly different from normal control group, P < 0.05
** Highly significantly different from normal control group, P < 0.001
DISCUSSION

Chicken pasteurellosis (fowl Cholera) is a serious, highly contagious disease caused by the bacterium *P. multocida* in a range of avian species including chickens. In the present study clinical signs showed depression, ruffled feather, drowsiness, diarrhea and loss of body weight, this result agrees with Amany (1997). The hemogram in the present study showed anemia as a result of decrease in RBCs count HB concentration and PCV. Such anemia could be attributed to hemolytic effect of the *P. multocida* endotoxin, Diallo and Frost (2000). The leukogram in gp 2 showed leucopenia due to heteropenia. Histopathological examinations of tissues revealed leucocytic aggregation in the liver and kidney. It is suggested that the observed heteropenia is due to shift of cells from the circulating pool into the marginal pool under the chemotactic effect of *P. multocida* endotoxin residing in tissues Howes et al., (1978). Treatment of infected chicken with gentamycin elicited non-significant changes in hemogram parameters. The significant increased values in AST and non-significant increase in ALT and GGT in gp 2 could be due to liver damage produced by the infected *P. multocida*. Campbell and Coles (1986) mentioned that the increased activity of AST has been associated with hepatocellular damage in chicken. Concerning ALT, some studies reported elevation of ALT in chickens in liver diseases (Bokori and Karasi, 1969). The increase in serum enzymes in the infected chicken could be attributed to the degenerative changes induced by bacteria or its endotoxins. The histopathological examination of liver in these gp showed degenerative changes in the hepatocytes. Our results is in agreement with Maglione (1962), Amany (1997) and EL-Sayed et al (2000). The increase values in uric acid in gp 2 during the experimental period. Uric acid is the main nitrogenous waste in avian species and the increased values could be due to the effect of the *P. multocida* microorganism or its endotoxin on the kidney. The histopathological examination of kidney in these gp showed degenerative changes in the epithelium of renal tubules. Our results is in agreement with Campbell and Coles (1986), Harrison and Harrison (1986) and Amany (1997).

Hypocalcemia seen in the present study could be due to decreased calcium resorption by the damaged renal tubules or associated with hypoalbuminemia as reported by Campbell and Coles (1986), Harrison and Harrison (1986) and Amany (1997).
phosphatemia could be due to renal disease. Also, the metabolism of calcium and phosphorus is closely linked in the body and hypocalcaemia is always accompanied by hyperphosphatemia (Campbell and Coles, 1986; Harrison and Harrison 1986 and Amany, 1997). The insignificant change in protein in the present studies may be due to the effect of endotoxin of \textit{P. multocida} on the hepatocytes. The decreased in A/G ratio could be due to the bacterial infection in chickens, which is in accordance with Omaima (1987) and Amany (1997). The encountered hyperglobulinemia with a decrease in albumin and A/G ratio was reported by Bierer (1969) due to \textit{Pasteurella multocida} infection. The electrophoretic separation of serum protein revealed a significant increase in values of beta and gamma globulin in gp 2 three and ten days PI.

The beta and gamma are the immune globulins. Elevations in the beta and gamma globulins usually indicates activation of the immune system and is most often due to infection or inflammatory diseases (Butler, 1983). Treatment of infected chicken with gentamycin showed non-significant changes in serum biochemical parameters. These revealed that gentamycin is active against \textit{P. multocida} (Booth, 1988).

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Mbuthia, P.G.; Njagi, L.W.; Nyaga, P.N.; Bebora, L.C.;


دراسات هيماتولوجية و بيوكيميائية ومناعية على الإصابة بالباستيريلا في الدواجن
سامية محمد
معمل بيطرى بني سويف – معهد بحوث صحة الحيوان

الملخص العربي
استخدم خمسة وأربعون دجاجة بلدى عمر اربعة أسابيع ثم تقسيمها إلى ثلاثة مجموعات. مجموعة (1) تتكون من 15 دجاجة استخدمت كمجموعة ضابطة. مجموعة (2) تتكون من 15 دجاجة تم حقن مجموعات 2 و 3 بالباستيريلا ملتوسيدا 2,0 مللي / طائر يحتوي على 10^4 ميكروب بستيريلا. تم علاج المجموعة الثالثة بعد ثلاثة أيام من العدوى بالجيتناميسين جرعة 4 مل/ كجم وزن الطائر لمدة 5 أيام. تم اخذ عينات من المجموعتين 2 و 3 بعد 2, 10, 17 يوم من العدوى ومن المجموعة 3 بعد اسبوع واسبوع من العلاج. اظهرت الدراسة نقص في عدد كرات الدم الحمراء و نسبة الهيموجلوبين و حجم الخلايا التكفي ونقص في عدد كرات الدم البيضاء. اظهرت الاختبارات البيوكيميائية زيادة في انزيمات ت. ا. س. ت. أ. ل. ت. ج. ج. ت. وزيادة في حمض البيوليك. اظهرت دراسة البروتينات نقص معنوي في الألبومين وزيادة في نسبة البيتا والجاما جلوبولين في المجموعة الثانية وأوضحت دراسة الأنسجة وجود احتقان في الكبد والأحشاء الداخلية.

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