قسم الباثولوجيا
كلية الطب البيطري - جامعة أسيوط
رئيس القسم: إبراهيم سالم

عدوى باستيريلا ملتوسيدة وباستيريلا أناتيستمر في البط
1- دراسات وبحثية

*عبد اللطيف بيومي، صلاح موسى، ناهد جاد
**عادل سليمان، محمد عطية

تم عزل عشرين ميكروب باستيريلا ملتوسيدة وكذلك ميكروب باستيريلا أناتيستمر.
تم تشخيص باستيريلا أناتيستمر على الآبار البيوكيميائية والбиولوجية أما عشرين باستيريلا
ملتوسيدة فتم تصنيعها على الآبار البيوكيميائية والبيولوجية والسيروجية.

تم استخدام العدوى الصناعية في البط بكم البكتيريا.

وأظهرت الزيئات المصابة بباستيريلا أناتيستمر النهايات في أنواع المهبط بالقلب والكبد في حين ظهرت الآثارية بميكروب باستيريلا ملتوسيدة على هيئة
أنزوع وتنكس الكبد والالتهابات رمادية.

* قسم أمراض الدواجن - كلية الطب البيطري - جامعة أسيوط
** قسم الميكروبولوجيا - كلية الطب - جامعة أسيوط
PASTEURELLA MULTOCIDA AND PASTEURELLA ANATIPESTIFER INFECTION IN DUCKS: I. EPIDEMIOLOGICAL STUDIES
(With 3 Tables & 2 Figs.)

By
A.H. BAYOUMI; S. MOUSA*; NAHED GAD*; A. SOLIMAN*
and M. ATIA**
(Received at 17/12/1987)

SUMMARY

Epidemics of duck pasteurellosis were diagnosed in duck farms at El-Wady El-Gadeed during 1985-1987. Pasteurella multocida (5tA and 2tD) and Pasteurella anatipestifer were isolated from different ages of ducks. Pasteurella anatipestifer was identified biochemically and biologically. Pasteurella multocida isolates were identified, biochemically, biologically and serologically. Successful reproduction of the disease was conducted with Pasteurella multocida and Pasteurella anatipestifer. Pasteurella anatipestifer infection resulted in serofibrinous inflammation in epicardium, pericardium and liver capsule. Pasteurella multocida infected birds showed haemorrhagic lesions, hepatic necrosis and interstitial pneumonia.

INTRODUCTION

Among the bacterial diseases of domestic ducks, Pasteurellosis accounts for major economic losses through high mortality, weight loss and condemnation.

Pasteurella anatipestifer (PA) is characterized by terminal pericarditis, pericarditis and airsacculitis, HEDDESTON, 1975.

Ducklings at 2-8 weeks old are most highly susceptible. Mortalities in natural outbreaks reached 75%, HEDDESTON, 1972.

Fifteen serotypes constituting (A-O) have been identified on the basis of agglutination reaction, HARRY, 1969 and HEDDESTON, 1975, while six serotypes have been differentiated by agar-gel precipitin (AGP) reactions, SANDHU and HARRY, 1981.

Infections with Pasteurella multocida usually occur in ducks over 4 weeks age with mortality more than 50%, DOUGHERTY, et al. 1955. Indirect haemagglutination test in conjunction with the serum agglutination test by NAMIOKA and BRUNER, 1963, who found 4 serotypes associated with fowl cholera.

** Dept. of Microbiology, Fac. of Vet. Med., Assiut Univ.
A.H. BAYOUMI, et al.

At El-Wady El-Gadeed, Egypt, duck pasteurellosis was observed as a persistent infection in different ages of duck flocks during 1985-1987, thus this investigation was undertaken to provide differential characteristics of the etiology and pathognomonic features of disease lesions.

MATERIAL and METHODS

History:
Outbreaks of fowl chiera in two white pekin duck farms at El Wady El Gadeed province were observed during 1985–1987. Involved flocks were of different ages, mortalities varied from 5–30% with higher rates usually observed in ducks of more than 4 weeks of age, while mild cases were recorded in younger birds. Dead cases were subjected to post mortem examinations. Smears and bacterial isolations were performed from liver and heart blood.

Flocks had a history of vaccination with a locally prepared monovalent (D, strain) bacterin.

Macro- and Micro-morphological examination:
Careful post-mortem examination was done on experimentally infected ducks, samples were taken in 10% buffered formaline, processed, stained with haematoxylin and eosin and examined histopathologically.

Bacteriological studies:
Isolation: Blood and MacConky agar plates were cultured from the heart blood and liver. Plates were incubated at 37°C for 24 hrs.

Blood agar plates were examined for non-haemolytic dew-drop like colonies. The colonies that revealed Gram negative bacilli or coccobacilli organisms were subcultured on another blood agar plates and tryptose broth tubes and incubated at 37°C for 18 hrs. Colonies were recultured on tryptose agar slope.

Identification:
a) Mice inoculation: 0.1 ml tryptose broth cultures were injected subcutaneously in 4–6 weeks old white mice, dead mice were inspected for recovery of the organism as well as detection of bipolar organisms in Gamsa stained smears from heart blood and liver and bacterial resolation.

b) Biochemical reactions: Simulating Pasteurella colonies were subjected to sucrose, marisol, glucose, and mannose sugars. Indol production, VP, MR, H₂S production, urase and gelatin liquefaction tests were performed after CRUICKSHANK, et al. 1975.

c) Serological identification: Slide agglutination test, NAMIOKA and MURATA, 1961, Agar-Gel precipitation test, HEDDESTON, 1975, were used for typing of isolates.

Experimental infection:
A total of 40 three weeks old ducklings and 40 eight weeks old ducks, each was divided into four equal groups. Birds were infected intramuscularly with 0.1 ml of 8 hrs. Pasteurella broth cultures as shown in table (3). Two groups served as non infected controls.

Infected and control birds were clinically observed. Dead and sacrificed birds were examined and samples were subjected to histopathological examination and bacterial resolation.
DUCK PASTEURELLOSIS

RESULTS

Liver and heart blood smears, stained by Giemsa stain taken from dead cases of different ages revealed the presence of bipolar, coccobacilli, intercellular organisms.

Out of 175 examined dead cases, 65 isolates of suspected Pasteurella organism were recovered, (Table 1). All isolates were gram negative bacilli or coccobacilli, and did not grow on MacConkey agar.

According to pathogenicity of white Swiss mice and biochemical reactions, isolates were grouped into two distinct groups, the first group, 41 isolates were lethal to mice, and glucose, manitol, mannose, sucrose, indol, H₂S production tests were positive, (Table 2). The second group, 24 isolates were non pathogenic to the mice, biochemically negative to glucose, manitol, mannose, sucrose, indol, H₂S, production, VP, MR tests. While urease test and gelatine liquefaction tests were positive (Table 2). This group appeared to be more closer to the side of P. anatipestifer.

Sero logically the first group of 41 isolates was identified as Pasteurella multocida 5*A (12 isolates) and (29 isolates) 2*D. Results of pathogenicity test of Pasteurella multocida serotypes 5*A and 2*D and Pasteurella anatipestifer are illustrated in Table 3.

On gross examination of the naturally infected cases, the lesions were restricted to the parenchymatous organs and the serous membranes. The parenchymatous organs including the liver, kidney, lung and spleen were swollen and severely congested. In few cases grayish, pinhead ed to few millimeter spots of necrosis were observed. In most cases the small intestines showed mild to severe catarrhal enteritis. The serous membranes involving the pericardium, the liver capsule, the pleura and air sacs thickened, turbid, and in many cases caseous, yellowish fibrin like deposits could be seen, Fig. 1. Subepicardial and skeletal muscle haemorrhages were prominent features in about 50% of the cases.

The P.anatipestifer experimentally infected birds showed haemorrhagic and septicaemic pictures. Serofibrinous inflammatory reactions in the serous membranes were constant findings. Histopathologically fibrinous epicarditis, pericarditis, and perihepatitis were observed. The exudate was laminated in ducks, profuse, mononuclear cell and fibrin rich on the liver of ducklings, Fig. 2. In the liver parenchymatous hepatitis associated with periportal mononuclear cell infiltrations could be seen. Subepicardial, endocardial and skeletal muscle haemorrhages were observed in young ages. In most cases the lungs showed interstitial mononuclear cell infiltrations. Peribronchial lymphoid hyperplasia could be also seen.

The post mortem and histopathological findings in the P.multocida infected ducks and ducklings were nearly similar. The subcutaneous tissues showed hyperaemia, oedema and mononuclear cell infiltrations. Petichial and ecchymitic haemorrhages on the epicardium could be observed. The liver showed focal areas of coagulative necrosis associated with hetrophile infiltrations. In the lungs, congestion, haemorrhages and mild interstitial pneumonia were constant findings.

DISCUSSION

Results of this investigation documents the susceptibility of white Pekin ducks of all ages to infection with P.multocida and P.anatipestifer.

According to the results of growth characteristics, pathogenicity to mice and biochemical reactions, isolates of \textit{P. multocida} (41 isolates) and (24 isolates) of \textit{P. anatipestifer} were distinguished.

Isolation of \textit{P. anatipestifer} from young, growing and adult ducks disagree with reports that ducklings at 2-8 weeks of age are most highly susceptible \cite{Hedlestone, 1972}.

It is worthy to conclude that \textit{P. multocida} was also isolated from young age ducklings as well as adult birds. On the other hand, Dougherty, et al. 1955 observed that \textit{P. multocida} usually occurred in ducks over 4 weeks of age.

Serologically, \textit{P. multocida} isolates were identified as 5A and 2D. Similar results were obtained by Carter and Bain, 1960 and El-Mongy, 1977, who found that type A and type D of \textit{P. multocida} were highly pathogenic to avian species. Regarding to the gross and microscopic findings, \textit{P. anatipestifer} infection resulted in serofibrinous inflammation in the serous membranes including the epicardium, pericardium and Glisson's capsule of the liver. Similar findings were reported, Dougherty, et al. 1955, and probably could be attributed to the increase in the vascular permeability. In \textit{P. multocida} infected birds, in addition to the circulatory disturbances observed, focal hepatic necrosis and interstitial pneumonia were seen, similar findings were also reported by Rhoades and Rimler, 1984.

**REFERENCES**


**DUCK PASTEURELLOSIS**

Table (1)

<table>
<thead>
<tr>
<th>Age of Examined dead birds</th>
<th>No. of Examined samples</th>
<th>No. of Positive isolates</th>
<th>Recovery Rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 4 weeks</td>
<td>75</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>4 - 8 weeks</td>
<td>60</td>
<td>26</td>
<td>43.3</td>
</tr>
<tr>
<td>More than 6 months</td>
<td>40</td>
<td>27</td>
<td>67.5</td>
</tr>
<tr>
<td>Total</td>
<td>175</td>
<td>65</td>
<td></td>
</tr>
</tbody>
</table>

Table (2)

Results of mice pathogenicity and biochemical reactions of recovered isolates

<table>
<thead>
<tr>
<th>Age of the Examined Birds</th>
<th>No. of Exam. isolates</th>
<th>Pathogenicity to mice</th>
<th>Growth on MacCoy's agar</th>
<th>Glucose</th>
<th>Mannitol</th>
<th>Mannose</th>
<th>Sucrose</th>
<th>Urease test</th>
<th>Gelatin liquefaction</th>
<th>Indol test</th>
<th>H₂ production</th>
<th>V.P. test</th>
<th>M.R. test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 4 weeks old</td>
<td>12</td>
<td>5</td>
<td>-</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4 - 8 weeks old</td>
<td>26</td>
<td>15</td>
<td>-</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>11</td>
<td>11</td>
<td>15</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>More than 6 months</td>
<td>27</td>
<td>21</td>
<td>-</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>6</td>
<td>6</td>
<td>21</td>
<td>21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>41</td>
<td>-</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>24</td>
<td>24</td>
<td>41</td>
<td>41</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table (3)

Results of Pathogenicity and Reisolation of inoculated Pasteurella organisms

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inoculated Organism</th>
<th>No. of inoculated birds</th>
<th>No. of dead birds</th>
<th>No. of positive reisol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 weeks ducklings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>5iA (P. multocida)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>II</td>
<td>2iD (P. multocida)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>III</td>
<td>P. anatipestifer</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>IV (Control)</td>
<td>--</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8 weeks ducks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>5iA (P. multocida)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>II</td>
<td>2iD (P. multocida)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>III</td>
<td>P. anatipestifer</td>
<td>10</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>IV (Control)</td>
<td>--</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

A.H. BAYOUMI, et al.

LIST OF FIGURES

Fig. (1): Showing thickening of pericardium, liver capsule and air sacs.

Fig. (2): Showing fibrinous perihepatitis.