

عدوى الباستيريلا ملتوسيدا وباستيريلا أناتيبستفر في البط
١ - دراسات وبائية

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- تم تشخيص أوبئة لمرض كوليرا البط في الوادي الجديد خلال سنوات ١٩٨٥ - ١٩٨٧ ،
تم عزل عترات ٥ أ ، ٢ لميكروب الباستيريلا ملتوسيدا وكذلك ميكروب باستيريلا أناتيبستفر
تم تشخيص باستيريلا أناتيبستفر على الاسس البيوكيميائية والبيولوجية أما عترات باستيريلا
ملتوسيدا فتم تصنيعها على الاسس البيوكيميائية والبيولوجية والسيرولوجية .
- تم استخدام العدوى الصناعية في البط بكلا الميكروبين .
- وأظهرت الطيور المصابة بميكروب باستيريلا أناتيبستفر التهابات فيرنييه على الاغشية
المحيطة بكل من القلب والكبد في حين ظهرت الاصابة بميكروب باستيريلا ملتوسيدا على هيئة
انزفة وتتركز الكبد والتهابات رئوية .

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**PASTEURELLA MULTOCIDA AND PASTEURELLA
ANATIPESTIFER INFECTION IN DUCKS:
I. EPIDEMIOLOGICAL STUDIES**
(With 3 Tables & 2 Figs.)

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SUMMARY

Epornitics of duck pasteurellosis were diagnosed in duck farms at El-Wady El-Gadeed during 1985-1987. *Pasteurella multocida* (5:A and 2:D) 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, perihepatitis and air sacculitis, HEDDLESTON, 1975.

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

Fifteen serotypes constituting (A-O) have been identified on the basis of agglutination reaction, HARRY, 1969 and HEDDLESTON, 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.

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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 cholera 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 (D₂-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.

Identifications:

- 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 Gemsa stained smears from heart blood and liver and bacterial reisolation.
- b) **Biochemical reactions:** Simulating Pasteurella colonies were subjected to sucrose, manitol, glucose, and mannose sugars. Indol production, VP, MR, H₂S production, urase and gelatin liquifaction tests were performed after CRUICKSHANK, et al. 1975.
- c) **Serological identification:** Slide agglutination test, NAMIOKA and MURATA, 1961, Agar-Gel precipitation test, HEDDLESTON, 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 reisolation.

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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 MacConky 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 liquifaction tests were positive (Table 2). This group appeared to be more closer to the side of *P. anatipestifer*.

Serologically 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, pinheaded 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 septicemic 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. Petechial and ecchymitic haemorrhages on the epicardium could be observed. The liver showed focal areas of coagulative necrosis associated with heterophilic 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*.

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

Isolation of *P.anatipestifer* from young, growing and adult ducks disagree with reports that ducklings at 2-8 weeks of age are most highly susceptible (HEDDLESTON, 1972).

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

Serologically, *P.multocida* isolates were identified as 5:A and 2:D. Similar results were obtained by CARTER and BAIN, 1960 and EL-MONGY, 1977, who found that type A and type D of *P.multocida* were highly pathogenic to avian species. Regarding to the gross and microscopic findings, *P.anatipestifer* infection resulted in serofibrinous inflammation in the serous membranes including the epicardium, pericardium and Glissons 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 *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.

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Table (1)
Shows the frequency of bacterial isolation

Age of Examined dead birds	No. of Examined samples	No. of Positive (No. of recovered isolates)	Recovery Rate %
1 - 4 weeks	75	12	16
4 - 8 weeks	60	26	43.3
More than 6 months	40	27	67.5
Total	175	65	

Table (2)
Results of mice pathogenicity and biochemical reactions of recovered isolates

Age of the Examined Birds	No. of Exam. isolates	Pathogenicity to mice	Growth on MacConky ap	Glucose	Manitol	Mannose	Sucrose	Urease test	Gelatine	Liquifaction test	Indol test	H ₂ production test	V.P. test	M.R. test
1 - 4 weeks old	12	5	-	5	5	5	5	7	7	5	5	-	-	
4 - 8 weeks old	26	15	-	15	15	15	15	11	11	15	15	-	-	
More than 6 months	27	21	-	21	21	21	21	6	6	21	21	-	-	
Total	65	41	-	41	41	41	41	24	24	41	41	-	-	

Table (3)
Results of Pathogenicity and Reisolation of inoculated Pasteurella organisms

Groups	Inoculated Organism	No. of inoculated birds	No. of dead birds	No. of positive reisol.
3 weeks ducklings				
I	5:A (P.multocida)	10	10	10
II	2:D (P.multocida)	10	10	10
III	P.anatipestifer	10	10	10
IV (Control)	--	10	-	-
8 weeks ducks				
I	5:A (P.multocida)	10	10	10
II	2:D (P.multocida)	10	10	10
III	P.anatipestifer	10	6	8
IV (Control)	--	10	-	-

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LIST OF FIGURES

- Fig. (1): Showing thickening of pericardium, liver capsule and air sacs.
Fig. (2): Showing fibrinous perihepatitis.



