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BACTERIOLOGICAL AND PATHOLOGICAL STUDIES ON *PASTEURELLA HAEMOLYTICA* IN DUCKS IN ASSIUT GOVERNORATE

(With 1 Table and 10 Figures)

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(Received at 4/10/2007)

دراسات بكتيرية وباثولوجية عن الباستيرلا هيموليتيكا فى البط
فى محافظة اسيوط

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لمعرفة تواجد ميكروب الباستيرلا هيموليتيكا فى البط تم أخذ عينات من (مائتان وخمسون) بطة من مزارع البط بمحافظة اسيوط وتم اجراء الفحص البكتريولوجي والاختبارات البيوكيميائية لعزل وتصنيف الميكروب. وكانت نسبة الإصابة بالميكروب 4% من الاعداد المفحوصة. تم وصف الصورة الاكلينيكية والتشريحية للطيور المصابة طبيعياً بالمرض والتي اظهرت فقدان الشهية وخمول واسهال مع أعراض تنفسية (كحة - رشح بالانف) مع التهابات بالرئة والتهاب الاكياس الهوائية واحتقان بالكبد مع وجود بقع تتركز. اخذت عينات من اعضاء البط النافق حديثا لاجراء الفحص الهستوباثولوجى الذى اظهر التهابات فى الكبد والكلى والرئتين والقلب ووجود خلايا بكتيرية بكثرة فى بعض القطاعات كما وجدت ايضا تغيرات فى الاوعية الدموية على هيئة تورم بين خلايا الكبد وسمك جدار الجيوب الكبدية وتغير جدار الوريد الاوسط لفصيص الكبد وايضا تتركز جزئى لصفيرة الكوبيبات بالكلى. اظهر اختبار الحساسية للمضادات الحيوية المختلفة على الميكروب المعزول أن الدانوفلوكساسين والجنتاميسين والتراميثوبريم هم الادوية الاكثر تأثيراً.

SUMMARY

A survey of the prevalence of *Pasteurella haemolytica* (*P. haemolytica*) in ducks in Assiut governorate was carried out on 250 birds from different duck farms. Bacteriological examination and biochemical tests revealed isolation of *P. haemolytica* at a rate of 4 %. Clinical signs of living ducks showed depression, loss of appetite, diarrhea and respiratory symptoms (coughing and a watery nasal discharge). Postmortem examination revealed pneumonia, airsacculitis and congestion of the liver with necrotic foci. Histopathologically Abundant amounts of bacterial organisms were present in some organs appeared as Gram negative

cocci in sections stained by Gram's stain. Liver, kidneys, myocardium and lungs showed inflammatory reactions together with vascular changes represented by edema, hyalinization of the central vein of hepatic lobules, thickening in the wall of hepatic sinusoids and segmental necrosis of glomerular tufts. The renal tubular epithelium was desquamated. The myocardium showed coagulative necrosis. The lungs showed atelectasis of many bronchioles and alveoli. In-vitro- sensitivity test revealed that danofloxacin, gentamycin and trimethoprim were the most effective drugs against isolated organism.

Key words: *P. haemolytica*, duck diseases, histopathology

INTRODUCTION

Pasteurella is a part of the family Pasteurellaceae, which comprises three genera: Pasteurella, Actinobacillus and Haemophilis. The most common species of the genus Pasteurella are: *P. multocida*, *P. haemolytica* or (*Mannheimia haemolytica*) *P. pneumotropica*, *P. uzeae*, *p. aerogenes*, *P. gallinarum*, and *p. anatipestifer*. *P. haemolytica* is rod-shaped, slightly pleomorphic with bipolar staining and a zone of hemolysis surrounds colonies of freshly isolated strains and a double zone of hemolysis on lamb's blood agar is characteristic (Ernst *et al.*, 1990).

Bailey and Scott's (1994) Stated that two different biotypes of *P. haemolytica* have been identified viz, biotype A and biotype T. They differ in a number of characteristics including pathogenicity, antigenic nature and biochemical activity. They also observed that *P. haemolytica* is heterogeneous and somewhat resembles *p. multocida* in that it has capsular and somatic varieties. The somatic antigens are so complex that serotypes are designated according to differences in capsular substances.

Edes *et al.* (1994) could isolate *P. haemolytica* from the intestinal content of Muscovy ducks aged 4 to 20 weeks which had enteritis. Postmortem examination revealed, hepatitis with necrotic foci. Louis *et al.* (2006) reported that Mannheimia (M) was proposed for the trehalose –negative [pasteurella] haemolytica complex including at least seven species. *P. haemolytica* was reclassified as: *M. haemolytica* and *M. glucosida* and most species of M are known as opportunistic pathogens.

The purposes of the present work are:

- Isolation and identification of *P. haemolytica* from ducks.
- Studying histopathological changes of internal organs of naturally infected ducks.

- In- vitro- sensitivity test of the isolated organism to some antimicrobial drugs.

MATERIALS and METHODS

Materials:

Specimens:

250 Samples (liver, lung, airsacs, kidneys, heart and intestine) obtained from apparently healthy and freshly dead ducks (5-12months old age) were collected from different private farms of ducks in Assiut governorate. They were subjected to postmortem and bacteriological examination

Media, reagents and solutions.

Brain heart infusion broth, yeast – tryptone broth, sheep blood agar plates, MacConkey's agar plates, urea agar base, semisolid agar, kovac's reagent, peptone water, gelatin liquefaction, oxidase, hydrogen peroxide 30%, maltose, glucose and sucrose.

Stain: Gram's stain

The different media and reagents used were prepared according to Cruickshank *et al.* (1975).

In- vitro- sensitivity discs used were:

Tetracycline (30 µg), ampicillin (10 µg), streptomycin (10 µg), trimethoprim (5 µg), danofloxacin (5 µg), gentamycin (10 µg), kanamycin (30 µg), penicillin G (10 µg), amoxycillin (25 µg), oxytetracycline (30 µg) and erythromycin (15 µg) .

Methods:

Isolation:

Samples from liver, lung, airsacs, kidneys and intestine were inoculated into brain heart infusion broth and yeast tryptone broth and incubated at 37°c for 18h., followed by subculturing on MacConkey's agar plates and sheep blood agar plates at 37°c for 24 h. Suspected colonies were examined for their colonial morphology (shape, color and size) and cellular morphology. Films stained by Gram's stain. Biochemical testes were carried out on the isolated strains. The characteristics of Pasteurella and related species of possible clinical significance were done according to methods for identification Ernst *et al.* (1990), Richard and Glisson (1997) and Glisson (2003).

Gross pathology:

Dead ducks were subjected for post mortum examination for the existence of gross pathological changes.

Histopathology:

Tissue sample from freshly dead birds were fixed in 10% neutral buffered formalin. Fixed tissues were dehydrated in series of alcohols, cleared in methyl benzoate and embedded in paraffin. Sections were cut and stained with HE. Selected tissue sections were stained with Gram's stain according to Bancroft and Stevens (1982).

In- vitro- sensitivity test:-

The determination of sensitivity of the isolated organism against different antibiotic discs was done according to Wettstein and Frey (2004)

RESULTS

Clinical signs of examined ducks showed: depression, loss of appetite, diarrhea and respiratory symptoms (coughing and a watery nasal discharge). Postmortem examination of ducks revealed pneumonia, airsacculitis and congestion of the liver with necrotic foci.

Bacteriological examination:

Bacteriological examination showed that the suspected colonies on sheep blood agar were surrounded by a single narrow zone of beta-hemolysis (*P. haemolytica* is the only pasturellae which form soluble haemolysin). On MacConkey's agar small pink colonies were formed. Turbidity was recorded in broth culture, later a granular deposit and pellicles are formed. The organism was non motile. Gram's stain showed small gram-negative rods or coccobacilli. Biochemical reactions revealed that suspected isolates of *P. haemolytica* were oxidase and catalase positive. The isolates ferment maltose, mannitol, glucose and sucrose. They were negative for indole, urease and gelatin liquefaction.

Bacteriological examination and biochemical reactions revealed isolation of *P. haemolytica* at rate of 4%.

Gross pathology:

Postmortem examination revealed that the livers were swollen, dark brown in color and with necrotic foci, mottled and congested kidneys. The heart showed presence of peteciae over the epicardium, lungs were congested. Airsacculitis were frequently observed in examined birds.

Histopathology:

Liver showed thickening in the wall of hepatic sinusoids, hyalinization of the central vein, edema and moderate to marked inflammatory cell infiltration in the centrolobular area and inbetween

hepatic cords. Inflammatory cell infiltration composed of lymphocytes, macrophages, epithelioid cells, heterophils, plasma cells and giant cells. (Fig. 1, 2 &3)

Kidneys showed hypercellularity of the glomerular tufts and proliferation of Bowman's capsule (Fig. 4). In some cases, the glomerular tufts showed segmental necrosis (Fig. 5). Abundant amounts of bacterial organisms were present in the glomerular arteriols and cortical tissue which appeared as Gram negative cocoobacilli in sections stained by Gram's stain (Fig. 4, 6&7). The renal tubular epithelium was desquamated (Fig. 8). The interstitial tissue occasionally contained fibrinous exudate. (Fig. 6).

The myocardium showed coagulative necrosis. Bacterial colonies were observed in between muscle fibers. Few numbers of lymphocytes and heterophils were present nearby bacterial colonies (Fig. 9).

The lungs showed atelectasis of many bronchioles and alveoli caused by accumulations of exudates composed of fibrin, lymphocytes, macrophages and plasma cells (Fig. 10).

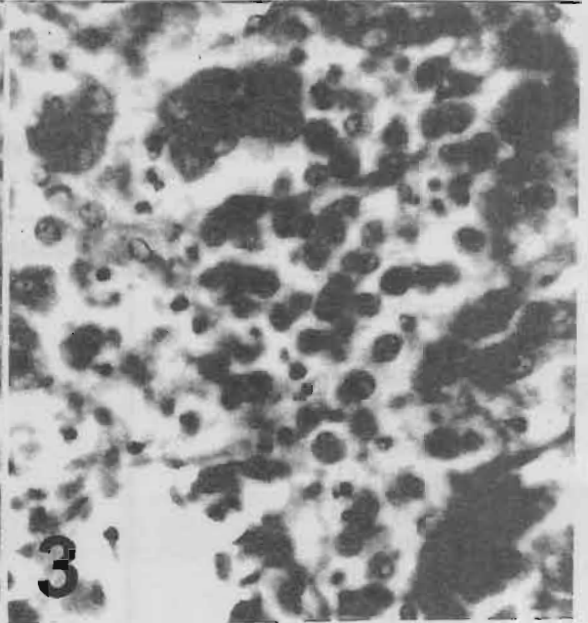
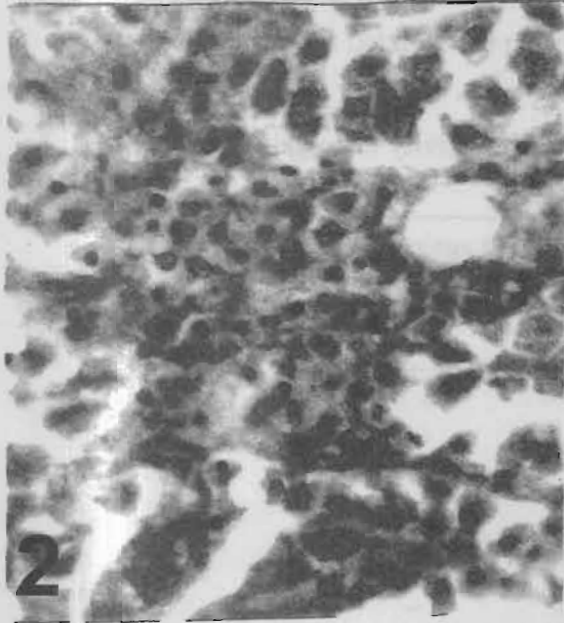
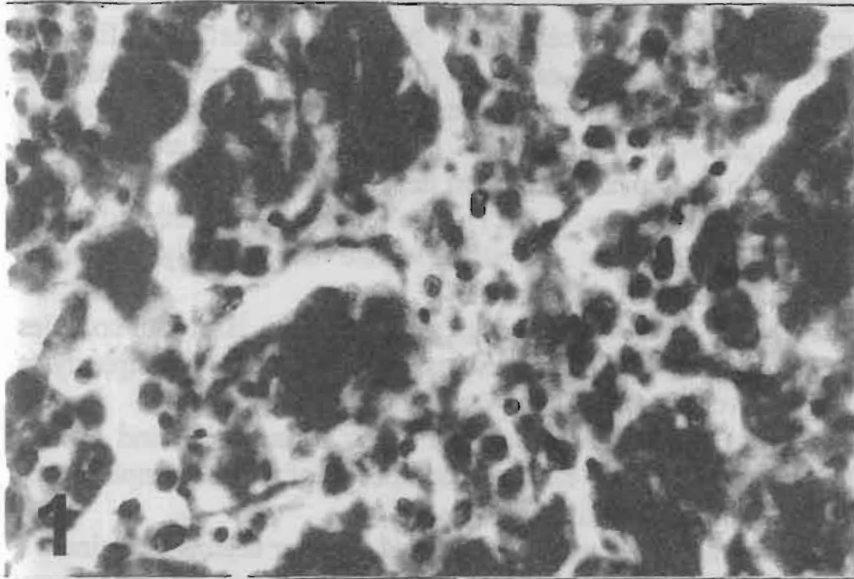
No remarkable lesions were observed in other organs examined.

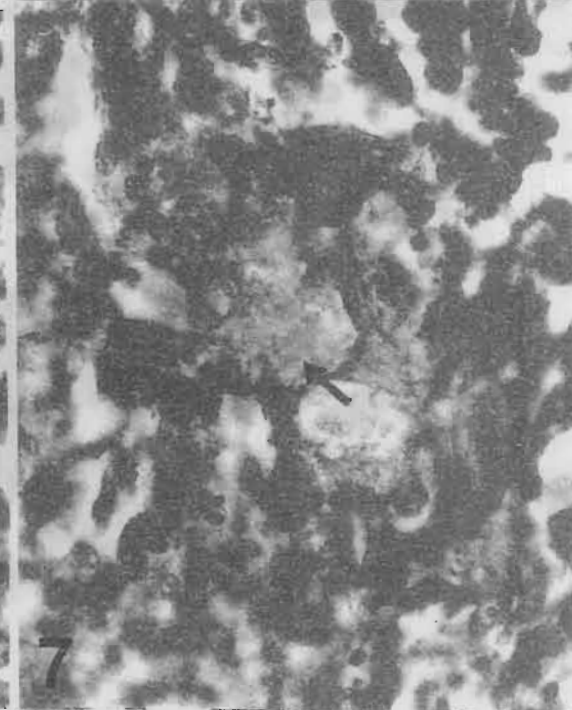
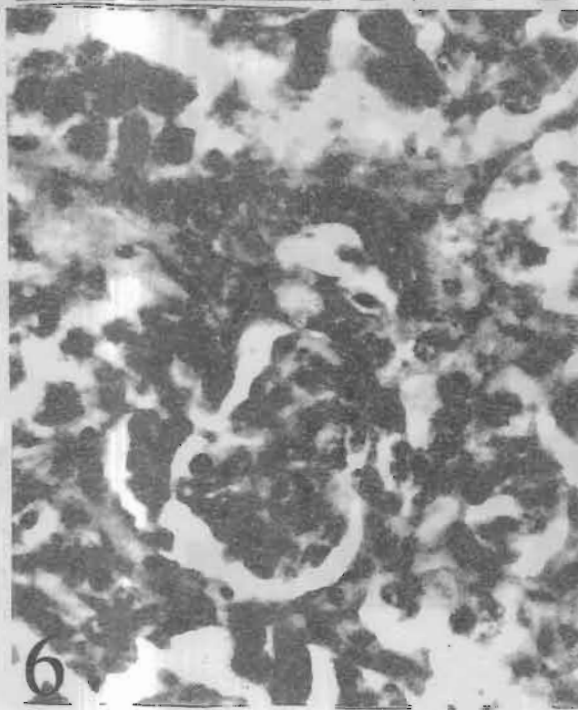
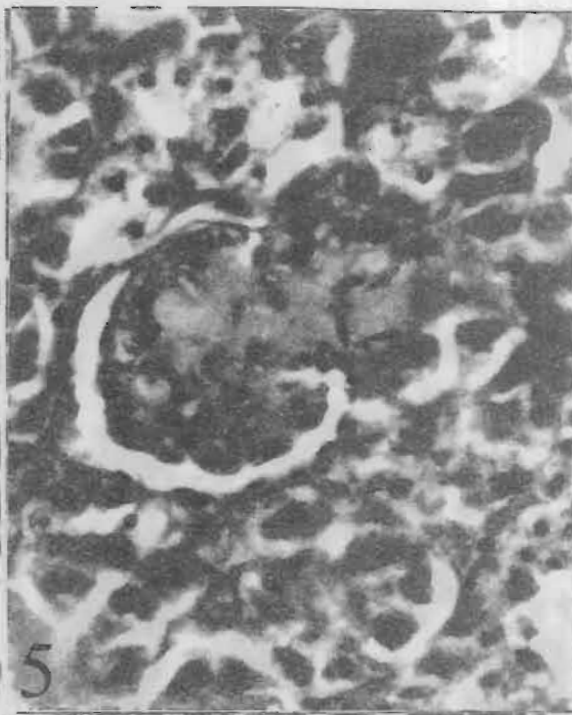
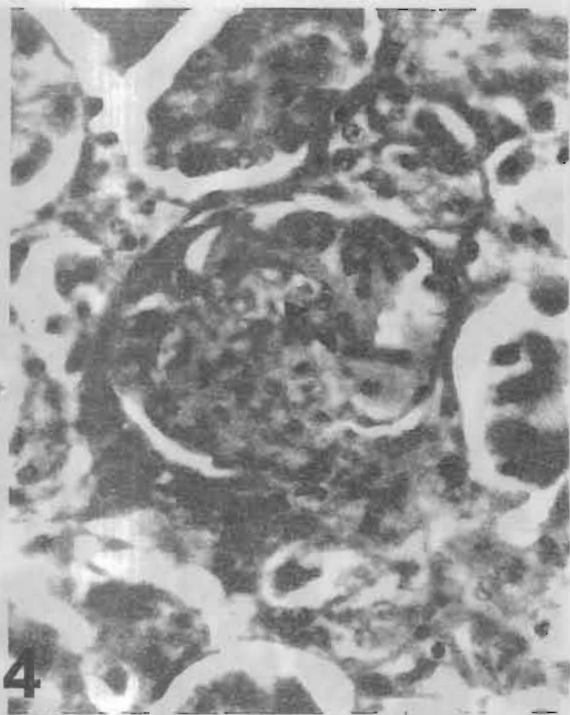
In vitro sensitivity test:

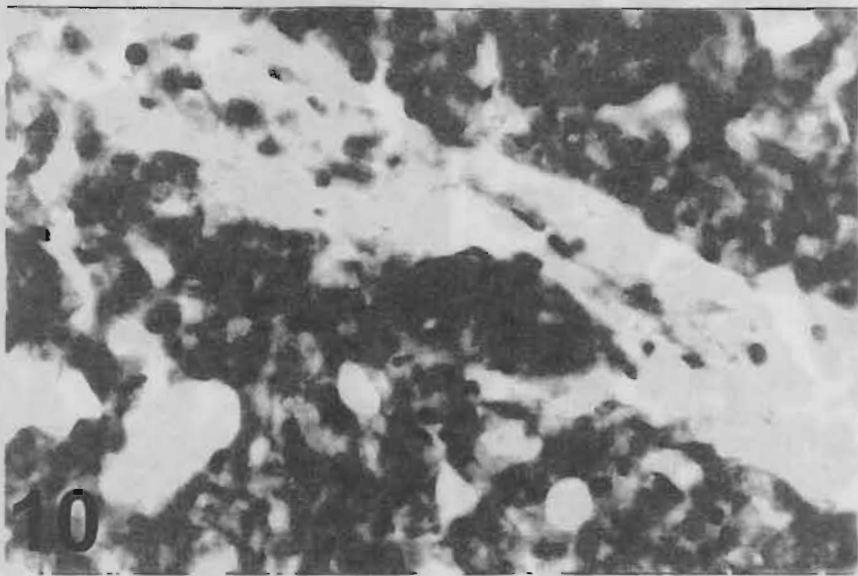
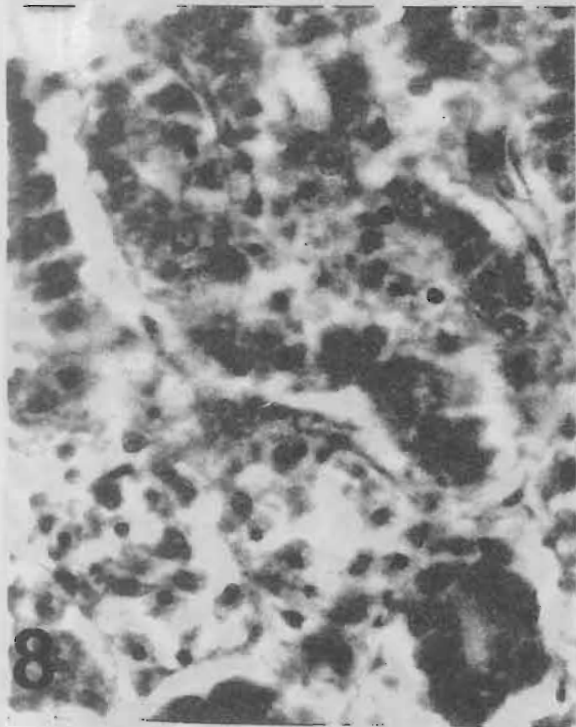
The effect of different antibiotics on the isolated *P. haemolytica* is illustrated in Table 1.

Table 1: illustrates the results of in vitro sensitivity test against isolated organism

Antibiotic discs	Sensitivity test against isolated organism
Danofloxacin	+++
Gentamycin	+++
Trimethoprim	+++
Oxytetracycline	++
Kanamycin	++
Penicillin G	++
Tetracycline	++
Erythromycin	+
Amoxycillin	-
Ampicillin	-
Streptomycin	-
+++ Sensitive	+weak sensitive
++ moderate sensitive	- resistant.







LEGENDS OF FIGURES

- Fig. 1:** Liver of a duck showing thickening in the wall of hepatic sinusoids, edema and inflammatory cell infiltration inbetween hepatic cords. HE. X400.
- Fig. 2:** Liver of a duck showing hyalinization of the central vein with marked inflammatory cell infiltration in the cetrolobular area. HE. X250.
- Fig. 3:** Higher magnification of the Fig.(2) showing marked inflammatory cell infiltration composed of lymphocytes, macrophages, epithelioid cells, heterophils, plasma cells and giant cells. HE. X1000.
- Fig. 4:** Kidney of a duck showing hypercellularity of the glomerular tufts, proliferation of Bowman's capsule and presence of bacterial organisms in the glomerular arteriols (arrows). HE. X1000.
- Fig. 5:** Kidney of a duck showing segmental necrosis of glomerular tufts. HE. X400.
- Fig. 6:** Kidney of a duck showing presence of abundant amounts of bacterial organisms in a cortical arteriole (arrow). The interstitial tissue contains fibrinous exudate. HE. X400.
- Fig. 7:** Kidney of a duck showing presence of abundant amounts of Gram negative cocoobacilli in the cortical tissue (arrow). Gram's stain. X1000.
- Fig. 8:** Kidney of a duck showing desquamation of the renal tubular epithelium. HE. X400.
- Fig. 9:** Myocardium of a duck showing coagulative necrosis, presence of bacterial colonies inbetween muscle fibers and few numbers of lymphocytes and heterophils infiltrated nearby bacterial colonies. HE. X400.
- Fig. 10:** Lung of a duck showing atelectasis of many bronchioles and alveoli caused by accumulations of exudates composed of fibrin, lymphocytes, macrophages and plasma cells. HE. X400

DISCUSSION

Pasteurella sp. is normal flora of the respiratory and gastrointestinal tracts of many species of domestic and wild birds so it has primary or secondary role in pneumonia of poultry (Bailey and Scott's 1994) and the virulence of *Pasteurella* is related to the Polysaccharide capsule that allows the organism to resist phagocytosis.

In this study, postmortem examination of freshly dead ducks showed pneumonia, airsacculitis, congestion of liver with necrotic foci. This result is some what similar to that reported by Edes *et al.* (1994) who found enteritis and hepatitis with necrotic foci.

Bacteriological examination revealed that *P. haemolytica* was recovered from lung, air sacs, kidneys, heart blood, intestine and liver. This result is partially similar to that observed by Edes *et al.* (1994) who isolated the organism from liver and intestinal content only.

The pathogenesis of *P. haemolytica* had explained by Yude Sun and Kenneth (1998) who stated that *P. haemolytica* is a commensal of the oropharynx and upper respiratory tract. It proliferates in the upper respiratory tract, subsequently colonizes the lower airway, produces various virulence factors and induces a profound inflammatory reaction resulting in pneumonic pasteurellosis. Leukocyte specific cytolytic leukotoxin (LKT) produced by *P. haemolytica* is considered to be a primary virulence factor. *P. haemolytica* LKT kills alveolar macrophages and neutrophils thereby diminishing the general response against bacterial infection in the lung.

In the present study, the inflammatory reactions in several organs including liver, kidneys, heart and vascular changes in the central vein of hepatic lobules and glomerular tufts may be attributed to various virulence factors produced by *P. haemolytica* (Yude Sun and Kenneth 1998). The presence of abundant amounts of gram negative coccobacilli in kidneys and heart tissues from cases positive for isolation of *P. haemolytica* in the present study together with presence of marked inflammatory in the several organs indicated that *P. haemolytica* causing acute septicaemias in ducks.

Vitro sensitivity test revealed that danofloxacin, gentamycin and trimethoprim were the most effective drugs. This result is in agreement with that observed by Norcia *et al.* (1999) and Gurbuz and Shain (2003). Wettstein and Frey (2004) found that *P. haemolytica* was resistant to tetracyclin, ampicillin and streptomycin while we found that our isolated organism was resistant to ampicillin, amoxycillin and streptomycin and showed weak sensitivity to tetracyclin.

It could be concluded that the *P. haemolytica* causing a highly serious disease in ducks resulting in economic losses.

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