Pathogenicity of *Aeromonas hydrophila* in chickens

By

**Mahmoud, A. M.*** and **Tanios, A. I.***

*Department of Pathology, Faculty of Veterinary Medicine, Cairo University.
**Sero**logy Unit, Animal Health Research Institute, Dokki, Giza.

**SUMMARY**

Seventeen isolates of *Aeromonas hydrophila* were isolated from 250 commercial broiler chicks with an incidence of 6.8%. Most *A. hydrophila* isolates (88.24%) were positive for exotoxin assay and congo red binding test, while 52.94% were positive for crystal violet binding activity. Most strains of *A. hydrophila* were sensitive to chloramphenicol, ciprofloxacin and norfloxacin followed by gentamicin and neomycin while nalidixic acid, tetracycline, streptomycin and trimethoprim-sulfamethoxazole had moderate effect. On the other hand, all *A. hydrophila* strains were resistant to amoxicillin, cephalothin, erythromycin and penicillin G. Two experiments were done. In the first experiment, chicks of one day old were infected with $1.5 \times 10^9$ organisms via subcutaneous and yolk sac. The infected chicks dead within 24 hr. *A. hydrophila* were isolated from most organs. The lesions observed included congestion in the internal organs and few cases showed hepatic and muscular petechiae. The ultrastructural study of this group showed presence of the bacilli inside the hepatocytes and macrophages with marked cellular changes. In the second experiment an attempt was made to determine a correlation between level of exposure and mortality. It was found that the mortality rate was relatively high (52.5%) after injection of a high dose ($3.5 \times 10^7$) of the organisms while it was (35%) in the low dose ($1.5 \times 10^9$). *A. hydrophila* was isolated from most examined organs. In the group treated with the low dose, marked degenerative and necrotic changes were observed in both hepatic and splenic tissue beside to muscular lesions manifested by hemorrhage, degeneration, oedema and myositis. In the group injected with the high dose, the lesions were more severe and characterized by diffuse areas of necrosis in hepatic tissue, thrombus formation in the blood vessels together with large number of bacterial colonies and bacilli in the hepatic tissue. Marked muscular necrosis and myophagia were also noticed. The ultrastructural study for this group showed heterocells and hepatocytes contain bacilli. In other cases, the bacilli were present in the phagosomes of phagocytic cells in the splenic tissue. Cytopathological lysis was common evidence in the examined cells.
INTRODUCTION

Aeromonas hydrophila (A. hydrophila) and other motile aeromonads (A. sobria and A. caviae) are receiving increasing attention as a human pathogens, especially as causative agents of gastroenteritis, wound infections and septicaemia (Janda and Duffey, 1988 and Marki et al., 2003). The microorganism occurs widely in nature, especially in water supply and variety of retail-level food (Palumbo and Buchanan, 1988).

The prevalence of A. hydrophila in avian species is indicated by studies that documented 10 isolations from 45 raptors (Needham et al., 1979) and 20 isolations from 15 species of 200, free-living, and companion birds (Shane et al. 1984). The organism is regarded as pathogenic as it was recovered as the only bacterial isolate from dead canaries, a toucan (Panigrahy et al., 1981), young poult (Gerlach and Bitzer, 1971) and cause enteritis in birds (Aguirre et al., 1992).

Akan et al. (1998) examined faecal and carcass samples of 351 chickens from 15 different flocks in Turkey. All of the 15 flocks were positive for motile aeromonads. A. hydrophila was the predominant species in both faeces and carcass samples. A. hydrophila was isolated from different ages of dead or sacrificed chickens, ducks and turkeys with percentages of 15, 22.5 and 20% respectively by Amal (2007) in Upper Egypt. Sarimehmetoglu and Kuplulu (2001) reported that broiler carcass and carcass parts have been contaminated to important level with motile Aeromonas species and it has been risk for public health. Furthermore, Glunder (2002) isolated A. hydrophila in nearly 3500 wild and pet birds provide statistically significant evidence that the composition of the intestinal flora may depend on dietary habits. The infection was found in 1.9% of the carnivorous and herbivorous species, in 7.1% of the omnivorous and in 12.4% of the carnivorous and insectivorous birds.

The broad spectrum of infection with A. hydrophila is paralleled by a range of virulence factors including adhesions, cytotoxins, haemolysin, and various enzymes. However, most strains of A. hydrophila produce enterotoxins, regardless of the source (Morgan and Wood, 1988). The presence of several genes encoding
for putative virulence factors and phenotypic activities that may play an important role in *A. hydrophila* infection (Escarpulli *et al.*, 2003). The pathogenicity of *A. hydrophila* to 5 and 17 day-old embryonated chicken eggs revealed mortality and hatchability rates ranged between (36.7 % and 30 %) and (63.3 % and 70 %) respectively (Youssif and Wafaa, 2003).

From pathological point of view, *A. hydrophila* is considered as one of the most important aquatic pathogens and usually infects different fish species causing severe pathological lesions including degenerative changes in hepatic and renal tissues with necrosis in severe infections together with ulcerative dermatitis (Cipriano *et al.*, 1984).

Although tissue reaction against *A. hydrophila* infection was studied in details at different aquatic species (Wang and Xu, 1985; Viola, 1991 and Yambot and Inglis, 1994), the available literatures did not cover the pathogenicity of this microorganism in chickens either in tissue or ultrastructural level.

Due to public health significance of *A. hydrophila* and little information regarding incidence, pathogenicity and ultrastructural cellular changes of *A. hydrophila* in chickens. The present work was undertaken to study the presence of *A. hydrophila* in chickens, the biochemical reactions of the isolates and the ability of the isolated strains to exotoxin assays, Congo red (CR) binding test, Crystal violet (CV) binding and antibiogram. Experimental infection of broiler chicks with the isolated *A. hydrophila* was done to study the post-mortem, histopathological and ultrastructural changes in this avian species in case of *A. hydrophila* infection.

**MATERIAL AND METHODS**

**Samples:**
Cloacal or faecal swabs were taken from 250 commercial broilers chickens apparently healthy prior to slaughter in different localities in Cairo, Egypt. Chickens originated from different flocks. Commercial sterile swabs incorporating a 1-ml ampoule of modified Stuarts bacterial transport medium were used to sample cloacal material or faeces. Samples were placed on ice, transferred to the laboratory, and examined within 3 hours of collection.

**Isolation and identification of *A. hydrophila***:
Each swab was transferred into 10 ml of sterile alkaline peptone water (APW, pH 8.4) and incubated for 18 hours for enrichment. Samples in APW were then plated on MacConkey agar medium and blood-ampicillin agar (BAA) containing 5 % sheep blood and 10 mg ampicillin per liter.
plates were incubated at 28°C for 24 h (Gray, 1984).

Colonies appear large, flattened and non-lactose fermenting with a defined zone of beta-haemolysis were selected and purified on the nutrient agar plates. Films from pure colonies were stained by Gram’s stain and examined microscopically. The identification of the isolates was carried by determining their morphological, cultural and biochemical characteristics according to criteria of Popoff (1984).

Exotoxin assay:

The heat-labile enterotoxin of *A. hydrophila* was detected in cell-free supernatant fluids by the suckling mouse assay (SMA). The fluid accumulation due to the action of enterotoxin is considered significant if there is an intestinal weight/body weight ratio greater than 0.03 (Burke et al., 1981).

Congo red (CR) binding test:

All *Aeromonas* isolates were tested for its growth status on Congo red medium. The reaction is best seen after 24 hours of incubation at 25°C and then left for additional 4 days. Congo red positive (CR+) was indicated by the development of bright or orange red colonies (Gray, 1984).

Crystal violet (CV) binding test:

All *Aeromonas* isolates were inoculated on trypticase soya agar and incubated 24 hours at 28°C, then flooded with a solution of 0.5 mg of crystal violet per ml, then the dye was removed after 2 minutes of contact (Panigua et al., 1990).

Antibiogram:

All *A. hydrophila* strains obtained in this study were tested for antibiogram using the disk diffusion technique as described by Finegold and Martin (1982). The following antimicrobial disks were used: amoxycillin 20 mg, cephalothin 30 mg, chloramphenicol 30 mg, ciprofloxacin 5 mg, erythromycin 15 mg, gentamicin 10 mg, nalidixic acid 30 mg, neomycin 30 mg, norfloxacin 10 mg, penicillin G 10 IU, streptomycin 10 mg, tetracycline 30 mg, trimethoprim/sulphamethoxazole 1.25/23.75 mg.

Experimental infection:

The isolates having the morphological, cultural and biochemical character of *A. hydrophila*, were subjected for pathogenicity test according to method described by Shane and Gifford (1984).

Experimental birds:

A total of one hundred and eighty White leghorn unsexed unvaccinated one day old chicks were obtained from commercial hatcheries. Water and commercial unmedicated balanced ration were given ad libitum. Twenty chicks were slaughtered and subjected for laboratory examination to be sure
that they were free from *A. hydrophila* infection.

**Preparation of inoculum:**

*A. hydrophila* isolates were grown in Brain heart infusion (BHI) broth for 18 hrs at 30°C. Pellets that were obtained by centrifugation of the BHI cultures at 2000 rpm for 15 min were resuspended in 3 ml of saline.

**Pathogenicity of *A. hydrophila***:

In first experiment, two groups of 20 two-days-old broiler chicks were inoculated, one subcutaneously and the other via the yolk sac, with 0.1-ml suspension of 1.5 X 10^9 organisms in saline per chick. Twenty uninoculated chicks served as controls.

In second experiment, two groups of 40 five-days-old chicks, were inoculated via the yolk sac with suspensions containing 1.5 X 10^9 or 3.5 X 10^7 organisms per chick, one dose per group. A third group of 20 chicks, which received a 0.1-ml injection of sterile saline per chick, served as controls. All chicks were kept for four weeks. All birds were examined daily for clinical abnormalities; autopsies were performed on chicks in moribund stage and subjected to bacterial, histopathological and electron microscope examination.

**Preparation of samples for pathological examination:**

Collected chickens in moribund stage from all groups were subjected to post-mortem examination and any lesion seen was recorded. Samples from the muscles, liver and spleen were taken, fixed in 10 % neutral buffered formalin, dehydrated in alcohol, cleared in xylol and embedded in paraffin. 4 m thick sections were prepared and stained with Hematoxylene and Eosin *(Carleton, 1976).*

**Preparation of samples for electronmicroscopy:**

The specimen from hepatic tissue of chicken group of 5 days old injected with high dose were fixed in 5 % cold cacodylate buffer gluteraldehyde (4°C 0.1 N, pH 7.2). Fixation was done by immersion of small pieces of such organ (1 mm x 1 mm) in gluteraldehyde. The tissues were fixed for 2 hours, washed by cacodylate buffer and then stored in the fixative solution at 4°C *(Carleton, 1976).* Further processing was carried out in the electron microscope unit of Assiut University – Egypt.

**Transmission electron microscopy:**

The tissue specimens were then washed in cacodylate buffer (pH 7.2) 3 - 4 times for 20 minutes each and then fixed in 1 % osmium tetraoxide for 2 hours. After that, they were washed in cacodylate buffer four times 20 minutes each. Ascending grades of alcohol (30, 50, 70, 90 & 100%) for dehydration of samples were used. After
dehydration, the samples were immersed in propylene oxide for 30 – 60 minutes then in mixture of propylene oxide and Epon mixture (1:1) for 30 – 60 minutes and finally in pure Epon mixture for 30 – 60 minutes. The samples were embedded in the poly-ethylene capsules containing the embedding mixture (Epon mixture and hardner). Tissue blocks were polymerized in an oven for 2 – 3 days at 60°C, by using LKB ultramicrotomes, semi-thin sections in thickness of 1 micron thickness were prepared. The sections were stained by toluedine blue method and examined by light microscope. For transmission electron microscope examination, ultra-thin sections were prepared by the same ultramicrotome in thickness of 500 A° then contrasted by uranyl acetate and lead citrate then examined by electron microscope JEM 10 c x 11, and photographed.

**RESULTS**

Seventeen isolates of *A. hydrophila* were isolated from 250 commercial broiler chickens with an incidence of 6.8 % (Table, 1).

Virulence attributes of isolated *Aeromonas* species from examined commercial birds were illustrated in Table (2). Most *A. hydrophila* isolates (88.24 %) were positive for exotoxin assay and congo red binding test, while 52.94 % were positive for crystal violet binding activity.

Most strains of *A. hydrophila* were sensitive to chloramphenicol, ciprofloxacin and norfloxacin followed by gentamicin and neomycin while nalidixic acid, tetracycline, streptomycin and trimethoprim sulphamethoxazole had moderate effect. On the other hand, all *A. hydrophila* strains were resistant to amoxicillin, cephalothin, erythromycin and penicillin G (Table 3).

All chicks infected with 1.5 X 10⁹ organisms via subcutaneous and yolk sac in the first experiment were dead within 24 hrs. Generally chicks died acutely without showing premonitory signs. No specific lesions were observed on postmortem examination, although generalized venous congestion was evident. *A. hydrophila* was reisolated from most organs (Table, 4).

In the second experiment, an attempt was made to determine a correlation between level of exposure and mortality. It was found that the mortality rate was relatively high (52.5 %) than in lower dose (35 %). *A. hydrophila* was isolated from most organs examined (Table 5). Infected chickens showed depression, ruffled feathers. The lesions observed included congestion in the most of the internal organs, few cases of hepatic petechiae and severely congested and unabsorbed yolk sac. No isolation or lesions were noted in control.
Table (1): Incidence of *A. hydrophila* from faeces of examined commercial birds.

<table>
<thead>
<tr>
<th>No. of examined birds</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>250</td>
<td>17</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Table (2): Virulence characteristics of *A. hydrophila* isolated from commercial birds.

<table>
<thead>
<tr>
<th>No. of tested <em>A. hydrophila</em></th>
<th>Exotoxin assay</th>
<th>Congo red (CR) binding</th>
<th>Crystal violet (CV) binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
</tr>
<tr>
<td>17</td>
<td>15</td>
<td>88.24</td>
<td>15</td>
</tr>
</tbody>
</table>

Table (3): Antimicrobial susceptibility of 17 *A. hydrophila* isolated from commercial birds.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>16</td>
<td>94.12</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>15</td>
<td>88.24</td>
<td>1</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>13</td>
<td>64.71</td>
<td>3</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>11</td>
<td>68.57</td>
<td>1</td>
</tr>
<tr>
<td>Neomycin</td>
<td>12</td>
<td>71.43</td>
<td>2</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>15</td>
<td>88.24</td>
<td>0</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>8</td>
<td>47.06</td>
<td>2</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>9</td>
<td>52.94</td>
<td>1</td>
</tr>
<tr>
<td>Trimethoprim / Sulphamethoxazole</td>
<td>9</td>
<td>52.94</td>
<td>1</td>
</tr>
</tbody>
</table>
Table (4): Mortality associated with *A. hydrophila* infection in 2-day-old chicks.

<table>
<thead>
<tr>
<th>Route of infection</th>
<th>Dose</th>
<th>Number</th>
<th>Mortality day</th>
<th>Cumulative total (%)</th>
<th>A. hydrophila isolation from organs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 2 3 4</td>
<td></td>
<td>Yolk sac Heart Liver Spleen Cloaca</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>1.5 X 10⁹</td>
<td>20</td>
<td>20 0 0 0</td>
<td>100</td>
<td>20/20* 20/20 20/20 20/20 17/20</td>
</tr>
<tr>
<td>Yolk sac</td>
<td>1.5 X 10⁹</td>
<td>20</td>
<td>20 0 0 0</td>
<td>100</td>
<td>20/20 20/20 20/20 20/20 16/20</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>20</td>
<td>0 0 0 0</td>
<td>0</td>
<td>0/20 0/20 0/20 0/20 0/20</td>
</tr>
</tbody>
</table>

* Number dead.
** Isolation of *A. hydrophila* / specimens examined of dead chicks.

Table (5): Mortality associated with 2 dosages of *A. hydrophila* administered via the yolk sac to 5-day-old chicks.

<table>
<thead>
<tr>
<th>Dose/organism</th>
<th>Number</th>
<th>Mortality day</th>
<th>Cumulative total (%)</th>
<th>A. hydrophila isolation from organs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5 6</td>
<td></td>
<td>Yolk sac Heart Liver Spleen Cloaca</td>
</tr>
<tr>
<td>1.5 X 10⁹</td>
<td>40</td>
<td>11 6 0 2 1 1</td>
<td>52.5</td>
<td>21/21** 21/21 21/21 20/21 18/21</td>
</tr>
<tr>
<td>3.5 X 10⁷</td>
<td>40</td>
<td>7 4 1 1 1 0</td>
<td>35</td>
<td>14/14 14/14 13/14 13/14 12/21</td>
</tr>
<tr>
<td>0 (control)</td>
<td>20</td>
<td>0 0 0 0 0 0</td>
<td>0</td>
<td>0/20 0/20 0/20 0/20 0/20</td>
</tr>
</tbody>
</table>

* Number dead.
** Isolation of *A. hydrophila* / specimens examined of dead chicks.
Pathological results:

In the experiment one (infected group of 2 days old chicks), the histopathological lesions were severe and characterized by diffuse necrosis in all parenchymatous organs. In the liver, the hepatic tissue showed loss of cellular details and massive necrosis of the blood vessel's wall in portal area (Fig. 1). The spleen showed diffuse necrosis where the tissue replaced by homogenous structurless necrotic areas (Fig. 2). In muscles, prominent oedema and myositis were common lesions. Oedematous fluid was accumulated between the muscle fibers in epimyceum and perimyceum together with mononuclear cells infiltration indicating myositis (Fig. 3). The electron microscope examination revealed presence of large number of bacterial bacilli in the cytoplasm of hepatic cells (Fig. 4). Evidence of nuclear destruction was noticed (Fig. 5). Mitochondrial swelling and phagosomes containing bacilli were also observed (Fig. 6). The observed bacilli in all examined cases in this group were not surrounded by any zone of lysis.

In the experiment two (infected group of 5 days old chicks), injected with high dose, the histopathological lesions were prominent, hepatic tissue showed congestion of the portal and sinusoidal blood vessels with focal areas of hepatocellular necrosis (Fig. 7) after 1 day post infection. Various stages of necrobiotic changes were prominent in the examined hepatocytes including karyopyknosis chromatolysis and karyolysis with evidence of cytoplasmic lysis. Kupffer cells of such specimens were markedly activated (Fig. 8). In spleen, prominent depletion of the lymphoid tissue was a common picture after four days post infection (Fig. 9). The lesions in muscular tissue at this time were characteristic where inter muscular oedema was noticed together with myolysis, myophagia and the characteristic bacterial bacilli were observed between muscle fibers (Fig. 10). At five and six days post infection, the lesions were closely similat to those at four days.

In the experiment two (infected group of 5 days old chicks), injected with low dose, the histopathological lesions were nearly similar to the previous group but in mild extent. Blood vessels congestion of hepatic tissue was a common picture while the hepatocytes showed prominent degenerative changes including granular and vacuolar degeneration. Some nuclei of hepatocytes appeared shrunken with prominent chromatolysis (Fig. 11). In spleen, necrobiotic changes was observed in the hemopoietic cells together with
heterophiles infiltration (Fig. 12). The lesions in the muscular tissue of this group was very clear. Prominent vascular and cellular inflammatory changes were observed. Muscular oedema, hemorrhage, congestion and inflammatory cells infiltration mainly heterophils were demonstrated (Fig. 13). Zenker's necrosis, myolysis and myophagia and heterophils infiltration was a common picture in this group (Fig. 14).

On the ultrastructural level, the hepatocytes of experimentally infected chicks in this group showed mitochondrial swelling and phagosomes containing bacilli (Fig. 15). The bacilli were also noticed inside the macrophage (Fig. 16). Cytopathological lysis of the hepatic cells was noticed with the presence of the organism (Fig. 17). The characteristic heterophils containing granules was also noticed closely related to hepatocytes containing bacilli (Fig. 18). Cytoplasmic degeneration of the hepatocytes was noticed in the cells contained bacilli of the microorganism (Fig. 19). In the infected cells with bacilli, a zone of cytoplasmic lysis was usually noticed surrounding the microorganism (Fig. 20).
Fig. (1): Liver of chicken in experiment one showed loss of cellular details and massive necrosis of the blood vessel's wall (arrows). H&E stain (X 200).

Fig. (2): Spleen of chicken in experiment one showed diffuse necrosis (arrow). H&E stain (X 200).

Fig. (3): Muscles of chickens in experiment one showed accumulation of Oedematous fluid between the muscle fibers in epimyceum and perimyceum together with mononuclear cells infiltration (arrows). H&E stain (X 200).

Fig. (4): Transmission electronmicrograph of hepatocyte of chicken in experiment one showed large number of bacilli in the cytoplasm without zone of lysis (arrow), (X 10000).

Fig. (5): Transmission electronmicrograph of hepatocyte of chicken in experiment one showed nuclear destruction (arrow), (X 10000).

Fig. (6): Transmission electronmicrograph of hepatocyte of chicken in experiment one showed mitochondrial swelling and phagosomes containing bacilli
Fig. (7): Liver of chicken in experiment two showed congestion of the portal and sinusoidal blood vessels with focal areas of hepatocellular necrosis (arrows). H&E stain (X 200).

Fig. (8): Liver of chicken in experiment two showed various stages of necrobiotic changes. Notice: karyopyknosis, chromatolysis and karyolysis (arrows). H&E stain (X 400).

Fig. (9): Spleen of chicken in experiment two showed prominent depletion in the white pulp of lymphoid tissue. H&E stain (X 200).

Fig. (10): Muscle of chicken in experiment two showed muscular oedema, myolysis. Notice the bacterial bacilli (arrows). H&E stain (X 400).
Fig. (11): Liver of chickens in experiment two showed granular and vacuolar degeneration. Notice: nuclei of hepatocytes appeared shrunken with prominent chromatolysis (arrow). H&E stain (X 400).

Fig. (12): Spleen of chicken in experiment two showed necrobiotic changes in hemopoietic cells together with heterophiles infiltration (arrow). H&E stain (X 400).

Fig. (13): Muscle of chicken in experiment two showed muscular oedema, congestion, hemorrhage and Inflammatory cells infiltration. H&E stain (X 200).

Fig. (14): Muscle of chicken in experiment two showed Zenker's necrosis, myolysis and heterophils infiltration (arrow). H&E stain (X 400).
Fig. (15): Transmission electronmicrograph of hepatocyte of chicken in experiment two showed mitochondrial swelling and phagosomes containing bacilli (arrow). (X 8000).

Fig. (16): Transmission electronmicrograph of macrophage of chicken in experiment two. Notice: The bacilli inside the phagosome (arrow). (X 10000).

Fig. (17): Transmission electronmicrograph of hepatocyte of chicken in experiment two showed cytopathological lysis of the cell (arrow). (X 10000).

Fig. (18): Transmission electronmicrograph of hepatocyte of chicken in experiment two showed the characteristic heterophils containing granules closely related to hepatocytes containing bacilli (arrow). (X 10000).

Fig. (19): Transmission electronmicrograph of hepatocyte of chicken in experiment two showed cytoplasmic degeneration of the hepatocytes the cells contain bacilli of the microorganism (arrow). (X 8000).

Fig. (20): Transmission electronmicrograph of hepatocyte of chicken in experiment two showed zone of cytoplasmic lysis surrounding the microorganism (arrow). (X 67000).
DISCUSSION

Increased awareness of Aeromonas species in animals and human has stimulated interest about possible existence and distribution among chickens in Egypt. The Aeromonas organism appears to be wide spread in nature, epidemiological studies have shown that it is present in water, fruits and vegetables (Martins et al., 2002 and Dumontet et al., 2003). At the same time Aeromonas species has been implicated in several outbreaks of food and water illness (Martins et al., 2002 and Borchardt et al., 2003).

In the present study, 17 isolates of A. hydrophila were isolated from 250 commercial broiler chickens with an incidence of 6.8% (Table 1). The faecal load of A. hydrophila appeared to be relatively low in examined chickens. The low incidence of A. hydrophila in examined chickens because samples were taken from apparently healthy birds prior to slaughter. This assumption is confirmed with Jindal et al. (1993) and Akan et al. (1998) who reported a similar low incidence of A. hydrophila in chickens. Stern et al. (1987) found a low incidence of Aeromonas spp. in livestock (pig, beef, sheep & turkey) faeces and suggested that many of food strains may not be of faecal origin. On the other hand, Amal (2007) isolated 45 isolates from 300 chickens (15%) of different ages in Upper Egypt. Also our result was disagreed with Sarimehmetoglu and Kuplulu (2001) whom isolated Aeromonas spp. from 116 (82.9%) of total 140 broiler carcases and carcasses parts purchased at different supermarkets in Ankara.

Although the prevalence of motile aeromonads in poultry faeces was found to be low, it was very high in carcass samples. These data suggested that during the slaughtering process motile aeromonads which originated from the intestinal contents of chickens spread to the carcasses via water used during processing (Akan et al., 1998).

Virulence attributes of isolated Aeromonas species from examined commercial birds were illustrated in Table (2). Most A. hydrophila isolates (88.24%) were positive for exotoxin. In this aspect, Krovacek et al. (1989) noted that the enterotoxin is highly produced by A. hydrophila and is responsible for the long term of diarrhoeal diseases. Higher results were obtained by Burke et al. (1982) who noted that 93% of A. hydrophila strains were enteropathogenic. Also, Turnbull et al. (1984) found 94.59% strains of Aeromonas species isolated from different sources were enterotoxi-
genic. On the other hand, lower enterotoxin production was reported by Singh and Sanyel (1992) found 56% of Aeromonas species have the ability to produce enterotoxin.

In the present study, most A. hydrophila isolates (88.24%) were positive for Congo red binding test (Table 2). Panigua et al. (1990) and Mona (2003) reported that all motile Aeromonas take up Congo red dye with different degree of colour from pale orange to deep red. There is no biochemical understanding of Congo red binding mechanism by bacteria but it is suggested to be associated with presence of B-Dglycan in the bacterial cell wall (Vinal, 1988).

In the present study, 52.94% A. hydrophila isolates were positive for crystal violet binding activity (Table 2). The obtained results agree with Panigua et al. (1990). Moreover Mona (2003) found the ability of isolated motile A. hydrophila species to bind Crystal violet were 64.7%.

In vitro determination of antimicrobial sensitivity test for local isolates of A. hydrophila is important in order to guide the choice of the most appreciated drugs required to produce a therapeutic effect. The obtained results recorded in Table (3) revealed that, most strains of A. hydrophila were sensitive to chloramphenicol, ciprofloxacin and norfloxacin followed by gentamicin and neomycin while nalidixic acid, tetracycline, streptomycin and trimethoprim sulfamethoxazole had moderate effect. On the other hand, all A. hydrophila strains were resistant to amoxicillin, cephalothin, erythromycin and penicillin G (Table 3). These results nearly similar to that reported by Rudin (1970) who found that most strains of A. hydrophila were highly sensitive to chloramphenicol, tetracycline, neomycin and streptomycin. Gado (1988) reported that gentamicin and chloramphenicol were the most antibiotics used against A. hydrophila. Amal (2007) also reported that gentamicin was the most effective drug (100%) while neomycin was moderately sensitive (80%). Kampfer et al. (1999) recorded that the most A. hydrophila isolates were highly susceptible to quinolones as ciprofloxacin and chloramphenicol.

In this study, all chicks infected with 1.5 X 10⁹ organisms via subcutaneous and yolk sac in the first experiment were dead within 24 h (Table 4). No specific lesions were observed on postmortem examination, although generalized venous congestion was evident. A. hydrophila was isolated from most organs. In this aspect, Shane and Gifford (1984) showed susceptibility of poult and chicks to exposure to A. hydro-
They reported that death occurred with 24 h in both 2-day-old chicks and 4-day-old poults, irrespective of the route of administration, indicating the virulence of the organism at the level of exposure.

In the second experiment, an attempt was made to determine a correlation between level of exposure and mortality. It was found that the mortality rate in birds injected with high dose was relatively high (52.5 %) than those infected with low dose (35 %). *A. hydrophila* was isolated from most organs examined (Table 5). The clinical signs observed were in agreement with those reported by El- Kashab (2001) and Amal (2007). The lesions observed included few cases of hepatic petechiae, venous congestion and unabsorbed and severely congested yolk sac. No isolation or lesions were noted in the control group. These results are in agreement with that reported by Shane and Gifford (1984) and Amal (2007).

In this study, it was clear that *A. hydrophila* infection in young chicks induced severe septicemic pathological lesions in hepatic, muscular and particularly hemopoietic tissues and so the microorganism is considered to be a cause for mortality problem in some chicken farms. In this concern, Shane and Gifford, 1984 and Shane et al., 1984 paid attention to prevalence and pathogenicity of *A. hydrophila* to different avian species including chicks, ducks and turkey poults. Water born infection could be the main source for infection in the farms.

In this study, the detected lesions could be attributed to the different virulence factors induced by the microorganism. *A. hydrophila* pathogenicity is the expression for exotoxin and endotoxin (lipopolysaccharide) production (Janda 1991).

In the ultrastructure study, the presence of bacteria within macrophages in hepatic tissue surrounded by hallow zone was agree with Easa et al. (1989). The zone of lysis considered as indicator for the ability of the microorganism to produce proteolytic enzymes (Mahmoud, 1996). The absence of lysis zone in experiment one may be time dependent because all chicks in this group died after 24 hours post infection.

The information given by the achieved results revealed that *Aeromonas* species (primarily a pathogen of aquatic biota) existed in the examined chickens and the microorganism induced pathological lesions in the internal organs. The effect of toxin products was detected bacteriologically and on ultrastructural level. Therefore, from our study, it was clear that *Aeromonas* species could be a
cause of serious disease problems in chicken farms and may play a significant role in the epidemiology of motile Aeromonas species infections. Finally, resolution of pathogenic mechanisms awaits further studies of Aeromonas species virulence markers.

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الأثر الممرض لميكروب الأوروموناس في الدماغ

محمد علي محمود، عادل إبراهيم طينوس
كلية الطب البيطري - جامعة القاهرة - قسم البيولوجيا
معهد بحوث صحة الحيوان - الدقى - الجيزة - وحدة السيرولوجي

الملخص العربي

يعتبر ميكروب الأوروموناس من الميكروبات التي تصيب الكائنات المائية على وجه الخصوص خاصة الأسماك وقد أثبتت بعض الدراسات أصابته الكائنات الأخرى بهذا الميكروب وتفاوت و معرفة الأثر الممرض لميكروب الأوروموناس في الدماغ تم عزل سبيكة عشرة عينة من ميكروب الأوروموناس وكانت نتيجة حضور 80 عينة من درجه التسمم التجاري بنسبة 88.24 عن 88% من المراقبات المعزولة كانت إيجابية عند فحصها لاختبارات إنتاج السم المخاطي و اختبار الارتباط بمادة أمهر الكوبونو بينما كانت الميكروبات المعزولة إيجابية اختبار النشاط الجلسي للبنفسجي البورى بنسبة 94.5% مما يدل على ضرورة استخدام الميكروبات المعزولة و إجراء اختبار الحساسية للمستعدهات الحيوية وجد أن معظم الع лидер المعزولة كانت حساسة للكلورامفيينكول، السبينفوكلاسين و السبينفولاسين على الترتيب. متوسطا بالجيتاميسين، النيترومين، حمض الكاليديسكيك، الاستراكلسين، و السطرياميسين بينما أثر التراب مينوتيوبي و السفاميتوكياسور تأثيرا طفيفا على الميكروبات المعزولة. و من ناحية أخرى فقد وجد أن جميع الع лидер المعزولة كانت مقاومة للمستعدهات الحيوية من أنواع الأوميكسينيلين، السيفاوتين، الأرتيراميسين والبنسيلين جي و الإثباتات الأثر الممرض للميكروبات المعزولة فقد تم عمل تجربتين في التجربة الأولى تم حقق مجموعتين من الدماغ عمر 40 يوم بجرعة 100 ميكيروبه تحت الجلد ومن طريقة كيس المح. أما التجربة الثانية فقد تم حقق مجموعتين من الدماغ عمر خمسة أيام من طريقة كيس المح بتركيز 100 ل 10، 30 ل 10 و 20 ل 10 على الترتيب. و بمتابعة الدماغ المعزول لوجود نقص كل الدماغ في التجربة الأولى بعد 4 ساعات من الحقن بينما كانت نسبة الوفيات في التجربة الثانية 70% للدماغ المعزول بجرعة 10، 30 ل 10 و 20 ل 10. و عند الفحص الظاهر للدماغ الذي نفق حديثا بعد العدوى التجريبية لوحظ ظهور احتقانات في الأعضاء الداخلية وفي حالات قليلة ظهرت نقط دموية على العضلات والكبد. و أثبت الفحص النسيجي حدوث نخر (تنكرز) في خلايا الكبد والطماع والعضلات بصورة متغايرة في المجموعتين وظهور المراحل المختلفة لتكرز الخلايا وكذلك حدوث إثبات عملية بينما ظهر الفحص بالتموكوبولوجي انتكروني وجود الميكروبات داخل الخلايا الأكولية للدماغ و خلايا الكبد مع حدوث تغيرات ثانوية في تركيبات السيتوبلازم تلك الخلايا وظهور هالات مذيلية في سيتوبلازم تلك الخلايا مما يدل على إنتاج تلك الميكروبات لسم محللة للأنسجة. ومن هذه الدراسة يمكن استخلاص أنه بالرغم من أن ميكروب الأوروموناس من الميكروبات التي تصيب الكائنات المائية إلا
أنه قد تم عزل ذلك الميكروروب من دجاج التسمين و أمكن إثبات الأثر الممرض لهذا الميكروروب في أنسجة الدجاج تجريبياً عن طريق الفحوصات البيئولوجية والبيولوغي و الفحص الدقيق بالميكروروب الإيبرجوري و لذلك فإنه من الممكن أن يكون هذا الميكروروب من مسببات بعض الأوبئة المرضية في مزارع الدجاج كما أنه من الممكن أن يكون للدجاج دور في وبائية و إنتشار هذا المرض. و ينصح بعمل دراسات مستقبلية تفصيلية لتوضيح هذا الدور.

المحكمون:
أ.د. سهير سكر
أ.د. ماجده محمد علي

أستاذ البايولوجيا - كلية الطب البيطرى - جامعة القاهرة
أستاذ أمراض الدواجن - كلية الطب البيطرى - منشور - جامعة بنها