

## PATHOGENICITY OF AEROMONAS HYDROPHILA INFECTION IN CHICKS

By

**Elham F. El-khashab**

Dept. of Poultry diseases Faculty of Veterinary Medicines Cairo University

### ABSTRACT

Chicks of two and five days old were experimentally infected with *Aeromonas hydrophila* via different routes: yolk sac (y.s), intramuscular (I/M), subcutaneous (S/C) and orally. The chicks were highly susceptible to infection. The mortality rate ranged from 60 to 100% according to the infective dose and route of infection. The postmortem lesions were characteristic. *A. hydrophila* can be reisolated from different visceral organs in variable degrees.

### INTRODUCTION

*Aeromonas hydrophila* (*A. hydrophila*) occurs widely in nature; in water (Picard *et al.*, 1983 and Kompanets *et al.*, 1992), in milk (Nagah - 1991), in meat (Ozbas *et al.*, 1996). Through coming short period the organism receive renewed interest as a human pathogen and has being isolated from cases of human diarrhea (Millership *et al.*, 1983 & Buchanan and Palumbo, 1985). In addition to gastroenteritis *A. hydrophila* infects human causing severe diseases such as osteomyelitis (Lopez *et al.*, 1968), meningitis (Qadri *et al.*, 1976), endocarditis (Davis *et al.*, 1978), skin infection (Joseph *et al.*, 1979), bacteremia (Riley *et al.*, 1996) and pneumonia (Takano *et al.*, 1996).

*A. hydrophila* has long been recognized as a pathogen of fish and frogs (Popoff, 1984; Son *et al.*, 1997 & Lehane, and Rawlin, 2000). The prevalence of *A. hydrophila* in avian species is indicated by studies that documented 20 isolations from 15 species of 200, free-living, and companion birds (Shane *et al.*, 1984). *A. hydrophila* was isolated from 80 out of 2236-birds. A mono infection was found in 4 cases while in all other cases the *A. hydrophila* infection was combined with enterobacteria and /or strepto- or staphylococci. Predisposing factors seems to be necessary to provoke outbreak of the disease (Glunder, 1988). *A. hydrophila* caused epidemic deaths of wild birds (Korbel and Kusters, 1989), and conjunctivitis in a pet parrot (Garcia *et al.*, 1992).

*A. hydrophila* also has been identified as a single etiological agent in diseases affecting different birds (**Panigraphy et al., 1981 & Ocholi and Kalejaiye, 1990**).

*A. hydrophila* has been identified as a pathogenic organism for chickens (**Shane et al., 1984**). The qualitative data of this pathogen in Egypt is lacking, therefore, the initial purpose of this investigation is to study the pathogenicity of local isolates of *A. hydrophila* in chicken.

## MATERIAL AND METHODS

### Chicks:

One hundred and twenty, one – day – old male LSL chicks were obtained from commercial hatchery. Ten out of these chicks were taken randomly, sacrificed and subjected to bacteriological examination to be sure that they were free from *A. hydrophila*. The remaining 110 chicks were used for experiments. The chicks were reared on flower, fed with commercial balanced ration.

### *Aeromonas hydrophila* strain:

Local isolate of *A. hydrophila* strain was formerly isolated from fish and identified by **Elham F. El-Khashab** (under press).

### Culture media: (for propagation and reisolation of isolate)

- Nutrient broth (oxid).
- Nutrient agar (oxid).
- Rimler-shots agar (R.S.) (**Shotts and Rimler, 1973**) the typical *A. hydrophila* colonies were round, dome – shaped yellow color on A.S. media.
- MacConkey agar (oxid).

### Experimental designs:

#### Experiment (1):

Five groups of 2 day old chicks, (10 each) were infected with 0.1 ml of *A. hydrophila*  $1.5 \times 10^9$  /1 in broth culture (**Shane and Gifford, 1985**) one dose for each groups, by different routs as following:

- G1 : Infected intra-yolksac (i/y)
- G2 : Infected intra muscularly (i/m)
- G3 : Infected subcutaneously (s/c)
- G4 : Infected orally (o.)
- G5 : Non infected control

The different groups were observed for ten days post infection for recording clinical signs, postmortem changes and mortality rate.

### Experiment (2):

Four groups of 5-day -old chicks, (15 each) were infected subcutaneously with incremental dosage of *A. hydrophila* as following (one dose for each bird):

G1 : Infected with 0.1 of  $3.5 \times 10^6$  / ml

G2 : Infected with 0.1 of  $2.0 \times 10^6$  / ml

G3 : Infected with 0.1 of  $1.5 \times 10^6$  / ml

G4 : Non infected control

Birds were observed for ten days post infection for recording signs and mortality rate. The reisolation of *A. hydrophila* from different organs were attempted.

### RESULTS

In the first experiment the infected chicks showed high mortality rate reached 100% in chicks infected via y.s., i/m and s/c routes but 60% in chicks infected orally as shown in Table (1) and Fig. (1).

Generally, chicks died acutely showing premonitory signs, while chicks that died late demonstrated a transitory period of depression characterized by ruffled feathers and pasty vent before death. Postmortem examination revealed generalized subcutaneous venous congestion as well as congestion of liver, spleen, lungs, intestine; especially duodenum showed severe hemorrhagic enteritis, liver also have streaks of hemorrhages as shown in photos. (1-8). The result of incremental doses in the second experiment showed 40% mortalities in first dose ( $3.5 \times 10^6$ ); 33.3% mortalities in second dose ( $2.0 \times 10^6$ ); 13.3% mortalities in the third dose ( $1.5 \times 10^6$ ) and 0% mortalities in non infected control as showing in Table (2) and Fig. (2).

The reisolation rate of *A. hydrophila* from the internal organs of dead chicks was different according to the route, dose and days post infection as shown in Table (3).

### DISCUSSION

In the first experiment the chicks showed high susceptibility to *A. hydrophila*. infection. Deaths occurred within 24 hours, irrespective of the route of administration except oral route; was 60%, while other routes were 100%. This high mortality rate accompanied with severe symptoms and characteristic postmortem lesions like congestion of internal organs and hemorrhages of duodenum and liver. These results agreed with **Shane and Gifford, (1985)**. Clinical diseases caused by this bacterium have usually been associated with stress, high environmental temperatures, or other factors that impair immune competence (**Panigrahy et al., 1981 & Ocholi and kalejaiye, 1990**).

In the second experiment the mortalities were varied with incremental dosage of *A. hydrophila*. It was 40% in dose of  $3.5 \times 10^6$ ; 33.3% in dose of

$2.0 \times 10^6$  and 13,3% in dose of  $1.5 \times 10^6$ . These results confirm that the infected dose of organism have great role in inducing the disease. This fact confirmed by **Shane et al., (1984) & Shane and Gifford, (1985)**. The reisolation of *A. hydrophila* from different organs of dead birds, were achieved from nearly all examined organs of chicks infected with high dose but differ in chicks infected with lower doses; the reisolation was failed from many organs like kidneys and lungs. These results agreed with **Shane and Gifford, (1985)**.

From the previous discussed data, it has been concluded that the local isolate of *A. hydrophila*. (which previously isolated from Fish and ducks) is highly pathogenic to chicks which must put in mind with its dangerous zoonotic effect of *A. hydrophila*. for human.

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**Table (1): Mortality rate of 2-days old chicks infected with *A. hydrophila*. at a dose of 0.1 ml of  $1.5 \times 10^9$  by different routes.**

| Group | Age | No. of birds | Route of Infect-ion | Pattern of deaths   |   |   |   |   |   |   |   |   |    | Cumulative Total | %  |     |
|-------|-----|--------------|---------------------|---------------------|---|---|---|---|---|---|---|---|----|------------------|----|-----|
|       |     |              |                     | Days post infection |   |   |   |   |   |   |   |   |    |                  |    |     |
|       |     |              |                     | 1                   | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |                  |    |     |
| G1    | 2d  | 10           | y.s                 | 10                  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0                | 10 | 100 |
| G2    | 2d  | 10           | I/M                 | 8                   | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0                | 10 | 100 |
| G3    | 2d  | 10           | S/C                 | 8                   | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0                | 10 | 100 |
| G4    | 2d  | 10           | Orally              | 0                   | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0  | 0                | 6  | 60  |
| G5    | 2d  | 10           | Control             | 0                   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0                | 0  | 0   |

**Table (2): Mortality associated with incremental dosage of *A. hydrophila* administrated S/C to 5days old chicks.**

| Group | No. | Dosage of A.H     | Deaths per days post infection |   |   |   |   |   |   |   |   |    | Cumulative total | % |      |
|-------|-----|-------------------|--------------------------------|---|---|---|---|---|---|---|---|----|------------------|---|------|
|       |     |                   | 1                              | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |                  |   |      |
| G1    | 15  | $3.5 \times 10^6$ | 3                              | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0                | 6 | 40   |
| G2    | 15  | $2.0 \times 10^6$ | 0                              | 2 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0  | 0                | 5 | 33.3 |
| G3    | 15  | $1.5 \times 10^6$ | 0                              | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0  | 0                | 2 | 13.3 |
| G4    | 15  | Control           | 0                              | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0  | 0                | 0 | 0    |

The mortality % increased with high dose.

**Table (3): Reisolation of *A. hydrophila*. from organs of dead chicks .**

| Days after infection | Dose of organisms        | <i>A. hydrophila</i> isolation from organs |             |       |      |        |        |
|----------------------|--------------------------|--|-------------|-------|------|--------|--------|
|                      |                          | Yalk sac                                   | Heart blood | Liver | Lung | Kidney | Cloaca |
| 1                    | 3.5x10 <sup>6</sup> (G1) | (A) 3/3                                    | 3/3         | 2/3   | 2/3  | 3/3    | 3/3    |
|                      | O (control)              | 0/2  | 0/2         | 0/2   | 0/2  | 0/2    | 0/2    |
| 2                    | 3.5x10 <sup>6</sup> (G1) | 2/2  | 2/2         | 2/2   | 2/2  | 2/2    | 2/2    |
|                      | 2.0x10 <sup>6</sup> (G2) | 2/2  | 2/2         | 2/2   | 2/2  | 2/2    | 2/2    |
|                      | O (control)              | 0/2  | 0/2         | 0/2   | 0/2  | 0/2    | 0/2    |
| 3                    | 3.5x10 <sup>6</sup> (G1) | 1/1  | 1/1         | 1/1   | 0/1  | 1/1    | 1/1    |
|                      | 2.0x10 <sup>6</sup> (G2) | 1/1  | 1/1         | 1/1   | 0/1  | 0/1    | 1/1    |
|                      | O (control)              | 0/1  | 0/1         | 0/1   | 0/1  | 0/1    | 0/1    |
| 4                    | 2.0x10 <sup>6</sup> (G2) | 1/   | 1/1         | 1/1   | 0/1  | 0/1    | 1/1    |
|                      | 1.5x10 <sup>6</sup> (G3) | 1/1  | 1/1         | 0/1   | 0/1  | 0/1    | 1/1    |
|                      | O (control)              | 0/1  | 0/1         | 0/1   | 0/1  | 0/1    | 0/1    |
| 6                    | 2.0x10 <sup>6</sup> (G2) | 1/1  | 1/1         | 1/1   | 0/1  | 0/1    | 1/1    |
|                      | O (control)              | 0/1  | 0/1         | 0/1   | 0/1  | 0/1    | 0/1    |
| 7                    | 1.5x10 <sup>6</sup> (G3) | 1/1  | 1/1         | 0/1   | 0/1  | 0/1    | 1/1    |
|                      | O (control)              | 0/1  | 0/1         | 0/1   | 0/1  | 0/1    | 0/1    |

A: + ve isolation *A. hydrophila*./ organs examined.

**NB:** The +ve reisolation *A. hydrophila* on *R.S media* with round, dome- shaped, yellow colonies.

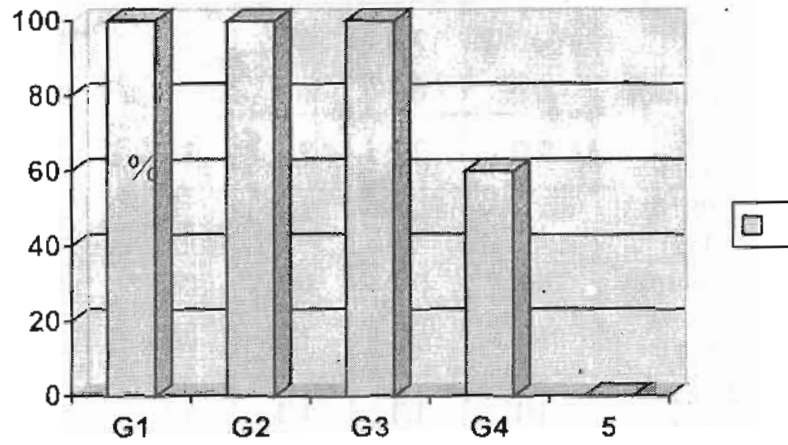


Fig. (1): Mortality % of 2-days old chicks infected with *A. hydrophila* at a dose of 0.1 ml of  $1.5 \times 10^9$  by different routes.

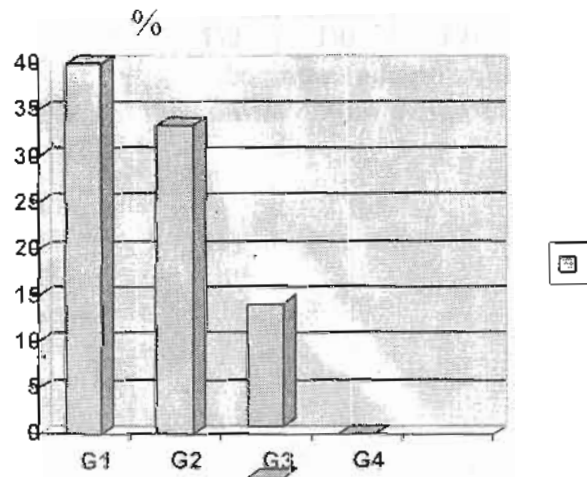
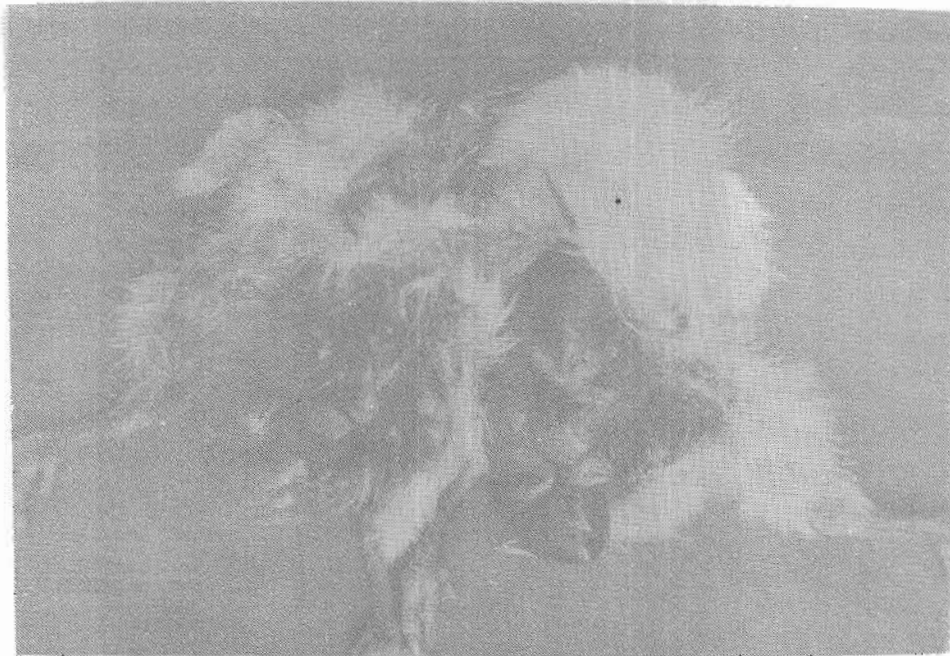


Fig. (2): Mortality associated with incremental dosage of *A. hydrophila* administered S/C to 5 days old chicks.





**Photo. (1)**



**Photo. (2)**

**Photos. (1-2): showing cogestion of all internal organs and yolk sac.**



**Photo. (3): Showing congestion of all internal organs and yolk sac.**



**Photo. (4): showing congestion of heart.**



**Photo. (5)**



**Photo. (6)**

**Photos. (5&6): Showing inflamed duodenum with hemorrhagic enteritis.**



**Photo. (7)**



**Photo. (8)**

**Photos. (7&8): Showing congestion of kidneys.**

## الملخص العربي القدرة المرضية للعدوى بميكروب الايرومونات هيدروفيليا في الكتاكت

إلهام فؤاد الخشاب

قسم أمراض الدواجن كلية الطب البيطري- جامعة القاهرة

تم دراسة القدرة المرضية للعدوى بميكروب الايرومونات هيدروفيليا في  
الكتاكت حيث تم عدوى الكتاكت في عمر ٢ و ٥ أيام بميكروب الايرومونات هيدروفيليا  
بطرق عدوى مختلفة : عن طريق كيس المح - في العضل - تحت الجلد - عن طريق الفم  
. هذا وقد أظهرت الدراسة أن للكتاكت قابلية كبيرة للعدوى حيث كانت نسبة النفوق عالية  
جدا حيث تراوحت بين ٦٠ و ١٠٠% تبعا لطريقة العدوى و الجرعة المعدية من  
الميكروب وكانت الآفات التشريحية مميزة للمرض كما تم إعادة عزل الميكروب من  
الأعضاء الداخلية المختلفة بدرجات متفاوتة .