Certain Epidemiological Aspects of *Aeromonas hydrophila* Infection in Chickens

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Abstract: *Aeromonas hydrophila* (*A. hydrophila*) is one of enteric poultry pathogens of public health importance. This work was designed to investigate certain epidemiological aspects of *A. hydrophila* including its viability, cycle of infection and its pathogenicity to chicks. A gentamicin resistant *A. hydrophila* strain (GR *A. hydrophila* strain*)* was prepared. The results showed that GR *A. hydrophila* survived in water for 26 days at room temperature and also it could be persist in chicken crates, feces, ration, saw dust and straw for 11, 9, 23, 22 and 17 days, respectively. GR *A. hydrophila* could induce 8.3% embryonic mortality after dipping of the eggs in infected broth culture. Hatched chicks from GR *A. hydrophila* infected eggs showed mortalities reaching 13.3 and 1.7 % during 1st and 2nd week post hatching, respectively. Survived infected chicks exhibited signs and lesions of omphalitis, enteritis and septicaemia and depression in heir weight gain. The rate of GR *A. hydrophila* re-isolation from dead embryos reached 100%, while it was 95.6, 26, 8.7, 4.4, 2.2 and 4.3% from intestine, liver, heart, spleen, kidney and lung, respectively in sacrificed survivors. Fecal shedding of GR *A. hydrophila* in chicken breeders revealed higher percentage in orally infected birds than subcutaneously infected ones. Addition of probiotic to the ration of orally infected group resulted in lowering the shedding rate. Re-isolation of the organism from egg shells reached 12 % in orally infected breeders compared to 4 % in orally infected probiotic treated birds. Samples taken from reproductive and internal organs of parent chicken hens were negative for GR *A. hydrophila* re-isolation. In conclusion; GR *A. hydrophila* survives for several weeks in contaminated water, ration and litter. The organism may infect birds by oral route and can colonize intestine. GR *A. hydrophila* is not congenitally transferred as ovary and oviduct do not play a role in dissemination of *A. hydrophila* infection. Addition of probiotic to the ration can reduce fecal shedding rate as well as re-isolation of *A. hydrophila* from the egg shells.

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Key Words: *Aeromonas hydrophila*, Chickens, Survival, Transmission

1. Introduction:

Aeromonas hydrophila (*A. hydrophila*) has been isolated from a wide range of mammals (Von and Zinterhofer, 1970), surface water and sewage (Hazen *et al.* 1978), fish and shell fish (Rippey and Cabelli, 1980), rabbits (Okewole *et al.* 1989 and Abdel-Gwad and Abdel-Rahman, 2004) as well as birds (Gerlach and Bitzer, 1971, Panigraphy *et al.* 1981, Glunder and Siegmann, 1989, Garcia *et al.* 1992, Jindal *et al.* 1993, FanDe *et al.* 1997, Akan *et al*. 1998, KeMin *et al*. 1998, Zeinab, 2007 and França *et al*. 2009). Moreover; *Aeromonas* species are considered as food born pathogens and of public health importance (Gracey *et al*. 1982, Altwegg, 1987, Altwegg and Geiss, 1989, Sarinehmetoglu and Kuplulu, 2001, Fukushema *et al.* 2007 and Chang *et al*. 2008).

A. hydrophila has a long survival rate in the environment (Araujo *et al.* 1991, Quinn *et al.* 1994, El-Khashab and El-Yazed, 2001 and Glunder, 2002).

Accordingly; this study was carried out in order to investigate certain epidemiological aspects of *A. hydrophila* including viability in drinking water and different chicken materials, cycle of infection as well as its pathogenicity to chicks.

2. Materials and Methods:

Aeromonas hydrophila strain:

A strain of *A. hydrophila* originally obtained from Animal Health Research Institute, Dokki, Egypt was used in this study. This strain has been isolated from an imported poultry meat meal and identified biochemically according to Bullock *et al.* (1971), Cruickshank *et al.* (1975), Popoff (1984) and Palumbo *et al.* (1985). For epidemiological investigation this strain has been rendered gentamycin resistant (GR *A. hydrophila*) using the method of Glunder and Siegmann, (1989) and Bisgaard *et al*. (1995) by successive subculturing in broth culture containing increasing quantities of gentamycin, starting with 2 µg/liter and ending by

100 mg/liter broth. This GR *A. hydrophila* strain proved to be able to grow on agar plates containing 100 mg gentamycin per liter.

Study on the viability of GR *A. hydrophila* in drinking water:

Viable bacterial cells of GR *A. hydrophila* was added to one liter of tap water in a rate of 5×10^9 /ml (pH 6.7) in plastic trough and kept at room temperature (25°C). Bacterial samples were taken at the following schedule; every 3 hrs during the first 12 hrs post inoculation (PI), every 6 hrs PI during next 72 hrs, every 12 hrs PI during 4th to 7th days, once daily during 8–14 days PI, twice a week during 15–21 PI, and weekly during 3–7 weeks PI. Culturing was done by adding one ml of tested tap water to 9 ml nutrient gentamycin broth (containing 100 mg gentamycin/ liter), incubated at 30°C for 24 hrs; then a loopful from resultant growth was streaked onto *Aeromonas* agar media as well as on MacConkey agar plate. The plates were similarly incubated for 24 hrs.

Study on the viability of *A. hydrophila* in different materials:

Sterile chicken crates, ration, feces, saw dust and straw were used in this experiment. Two hundreds grams of each sample were inoculated with 5×10^9 viable bacterial cells of GR *A. hydrophila* broth culture per each gram then kept at room temperature (25°C). Samples were taken frequently in the following manner; day 1-3 PI twice daily, day 4-7 PI daily, day 8-23 PI every other day and day 24-45 PI weekly. Collected samples were inoculated into nutrient gentamycin broth, incubated at 30°C for 24 hrs then a loopful was streaked onto *Aeromonas* agar media as well as on MacConkey agar plate. The plates were incubated at 30°C for 24 hrs.

Study on the effect of dipping embryonated chicken eggs in *A. hydrophila* broth culture:

This method was done according to Zeinab *et al.* (2011). Eighty five, 18 day-old embryonated chicken eggs (ECEs) were divided into 2 groups (1 and 2). Those of group 1 were consisting of 60 eggs while those of group 2 were consisting of 25. Eggs of the 1st group were infected with GR *A. hydrophila* by dipping in 18 hrs chilled broth culture containing 6.1×10^9 CFU/ ml for five minutes. Those of the 2nd group were similarly dipped in sterile nutrient broth as a control. ECEs of both groups were further incubated with daily observation for embryo livability or mortality. Specimens including yolk sac, liver, heart and intestine of dead embryos were collected and subjected to bacteriological examination for GR *A. hydrophila* re-isolation. Liver was taken from dead embryos for histopathological examination.

Hatched chicks from both groups were kept separately for 21 days with close observation for clinical signs and mortality. The body weight was taken weekly. Sacrificed survived chicks at the end of observation period were necropsied and the organs including intestine, liver, heart, spleen, kidney and lung were subjected for bacteriological examination in an attempt of GR *A. hydrophila* re-isolation. From dead as well as sacrificed chickens at the end of study (21 day old), specimens from liver, heart, intestine and lung were collected for histopathological examination.

Study on the cycle of *A. hydrophila* infection:

Experimental design:

Thirty-four; 33 week-old chicken breeders consisting of 30 hens and 4 cocks were assigned randomly into 4 groups (1-4). Those of groups 1-3 were consisting of 8 hens and one cock while the 4th group was consisting of 6 hens and one cock. Each chicken of groups 1 and 2 were orally inoculated with 2 ml of GR *A. hydrophila* containing 1.5×10^9 CFU/ml. Chickens of group (1) were fed on a ration containing 0.5 kg / ton of a probiotic premix of selected lactic acid bacteria containing 10^9 CFU/g. of *Pediococcus acidilacti* produced by Lallemand Co.; France under the trade name Bactocell®, batch No. 402060 during the entire period of the experiment. Those of group (2) were fed on a plain ration without a probiotic. Chickens of group (3) were subcutaneously inoculated at the back of the neck with one ml/bird with GR *A. hydrophila* containing 1.6×10^9 CFU/ml. Birds of group (4) were kept without infection or treatment as a blank control group. At the 3rd day PI; cloacal swabs were collected from each group daily during the 1st week PI and every other day during 2nd week PI to study the fecal (cloacal) shedding of GR *A. hydrophila*. Fertile eggs were collected for GR *A. hydrophila* re-isolation. At the end of the experiment, all parent chickens were sacrificed and specimens were collected from ovary, intestine, heart, liver, spleen, kidney, lung, brain and different parts of oviduct and subjected to bacterial re-isolation.

1. Re-isolation of GR *A. hydrophila* from fertile eggs:

One hundred fertile eggs were collected from the infected as well as non infected breeder hens (25 eggs/ group). These eggs were subjected to bacteriological examination for re-isolation of GR *A. hydrophila* from egg shell as well as from egg albumin and egg yolk after Shane and Gifford (1985) as follows: The eggs were stored for 5 days at 4°C before the outer shell and the internal egg contents

were cultured for GR *A. hydrophila*. For outer egg shell examination, the eggs were placed for 5 minutes in nutrient broth in sterile plastic bags and the broth was incubated at 30°C for 24 hrs before streaking on gentamycin *Aeromonas* agar and gentamycin MacConkey agar media. For internal egg examination, the yolk was cultured by swabbing the pointed end of the egg with 70% alcohol, puncturing the shell with a sterile forceps to drain out albumin without breaking the vitelline membrane. The vitelline membrane was then cut with sterilized scissors and 1 ml of yolk was collected with a syringe and incubated at 30°C for 24 hrs in 15 ml of nutrient broth before streaking on gentamycin *Aeromonas* agar and gentamycin MacConkey agar media.

2. Re-isolation of GR *A. hydrophila* from breeder hens:

After 2 weeks observation period, all chicken breeder hens groups (1-4) were sacrificed and specimens were collected from ovary, intestine, heart, liver, spleen, kidney, lung, brain and different parts of oviduct (infundibulum, isthmus, uterus and vagina). The collected samples were subjected to bacteriological examination for determination of localization sites of GR *A. hydrophila.* Sampling of organs on GR *A. hydrophila* was done as follows: yolk swabs collected from the interior of ovules after the exterior was sterilized by searing with spatula was placed into 10 ml of nutrient broth. The exterior of the oviduct was seared at the junction of the magnum and isthmus and 5-6 ml of nutrient broth was injected into the lumen. The posterior end of the oviduct was lifted slightly so that broth transferred almost the entire length of the magnum. After 5-10 minutes the content (2-3) ml from magnum were poured into tube containing 5-6 ml of nutrient broth. Similarly the exterior of the liver, spleen, heart, kidney, lung and brain were seared and their interiors were sampled in sterilized swabs then cultured. Moreover; contents of the caecum were also cultured. All specimens were cultured on nutrient broth and incubated at 30°C for 24 hrs then streaked on gentamycin *Aeromonas* agar and gentamycin MacConkey agar media.

Histopathological Examination:

Specimens including liver, heart, intestine and lungs were collected from dead embryos as well as sacrificed hatched chicks, fixed in 10% formol, embedded in paraffin, sectioned and stained by hematoxylin and eosin stains (Banchroft *et al*. 1996) for histopathological examination through light microscope.

3. Results and Discussion:

The clinical significance of *A. hydrophila* was reported in several species of poultry as it caused septicaemia in turkeys (Gerlach and Bitzer, 1971), salpingitis in ducks (Bisgaard *et al*. 1995), diarrhea in water fowl (Efuntoye, 1995), conjunctivitis in pet parrots (Garcia *et al*. 1992), weight loss and diarrhea in cockatiels and canaries (Rosskopf and Woerpel, 1996) and diarrhea, feathers picking, sleeping, growth retardation and fluffing in different avian species (Jindal *et al*. 1993, Dorrestein, 1997 and Ahmed 2004). *A. hydrophila* can cause localized or systemic infections in different avian species either alone or combination with other microorganisms (Barnes 1997).

As *A. hydrophila* is sensitive to gentamicin (FanDe *et al*. 1997, San *et al*. 1997 and Kelley *et al*. 1998), a gentamicin resistant *A. hydrophila* strain *(*GR *A. hydrophila* strain*)* was prepared for labeling purpose during the present investigation.

The viability of *A. hydrophila* in tap water was investigated under controlled laboratory conditions. The organism survived in water for 26 days at room temperature (25°C). This finding can explain why *A. hydrophila* organisms were isolated from water samples in high percentage during winter season. Opposite result was recorded by Rippey and Cabelli (1980) who found that *A. hydrophila* seemed to be seasonally distributed with maximum count during summer through early fall and this may be due to that the examined sample in this work was tape water which of low faecal pollution (Araujo *et al*. 1991). The association of *A. hydrophila* with water and fish (Schubert *et al*. 1972, Austin, 1987 and Humphrey *et al*. 1987) and also its isolation from wild birds (Glunder and Siegmann, 1989) confirmed the long survival time of *A. hydrophila* in water which might result in the spread of infection within the flock. Our results agree with Kaper *et al*. (1981), Burke *et al*. (1984a, b), Arcos *et al*. (1988) and Varnam (1991) who reported on the isolation of *A. hydrophila* and other *Aeromonas* spp. from unchlorinated water supply. Legnani *et al*. (1998) studied the occurrence of *Aeromonas* spp. in drinking water supplies in a mountain area in northeast Italy as most of the isolates were identified as *A. hydrophila* and they suggested search for these micro-organisms should be adopted as a further indicator of drinking water quality. Also, Martone-Rocha *et al*. (2010) isolated *Aeromonas* spp. from wastewater treatment system.

The viability of GR *A. hydrophila* was investigated also in different material, simulating the flock condition to predict the mechanism of spread. Our findings showed that *A. hydrophila* persisted in chicken crates, feces, ration, saw dust and straw for 11, 9, 23, 22 and 17 days, respectively. Reviewing

the available literature, scanty literature reported on the viability of *A. hydrophila* in the previously mentioned materials. Rosskopf and Woerpel (1996) found that birds were usually exposed to infection with *A. hydrophila* through their food and transmission is primarily by oral routes with fecal shedding into environment. On the other side, Kelley *et al*. (1998) isolated *A. hydrophila* and other bacteria during the microbial evaluation of coarse fraction of litter for its reutilization as a bedding supplement in growing flocks of broilers.

The mortality rate of embryos and hatched chicks taken from GR *A. hydrophila* infected eggs and control non infected ones is shown in Table (1). The embryonic mortalities were 8.3% in GR *A. hydrophila* infected group as compared with 0% in non-infected control. This indicated the responsibility of *A. hydrophila* for inducing hatchability rate 91.7%. This finding assumed the possibility of transmission of GR *A. hydrophila* via egg shell penetration. This finding supported the results of Zeinab *et al.* (2011). Increased humidity and temperature as well as poor hygienic hatchery conditions are incriminated in provoking *A. hydrophila* infection via egg shell penetration. Musgrove *et al.* (2008) isolated *A. hydrophila* and other enterobacteria from the eggs shell of chickens. Dead embryos exhibited severe congestion of the liver, myocardium and yolk sac. Moreover, hatched chicks from GR *A. hydrophila* infected eggs showed mortalities reaching 13.3 and 1.7 % during 1st and 2nd week post hatching, respectively as compared with 0% in non-infected control. Survived infected chicks exhibited omphalitis, ruffling feathers, general weakness, inappetance and enteritis. At necropsy; hatched survivors from GR *A. hydrophila* infected eggs revealed enteritis, omphalitis, unabsorped yolk sac, distended gall bladder and congestion of liver and heart. Gerlach and Bitzer (1971) described septicaemic condition in commercial turkeys aged 3-16 weeks that was attributed to *A. hydrophila* infection with 10-30% morbidity rate and 1-5% mortality rate. The synergistic relationship between Salmonella spp. and *A. hydrophila* infections in newly hatched poults was studied by Saif and Busch (1974) who found that both organisms together produced 30% mortality but neither of them produced mortality when inoculated individually. Furthermore, Shane and Gifford (1985) reported that 2-4 day-old experimentally infected chicks were highly susceptible to *A. hydrophila* exposure via subcutaneous, yolk sac or intracerebral routes with mortality rate ranging 80-100%. Glunder (1988 and 1989) isolated *A. hydrophila* from 80 birds from a total of 2236 purchased birds. He found that mono infection was found in 4 cases while in all other cases, *A. hydrophila* infection was combined with the presence of Enterobacteriaceae and/or *Streptococci* or *Staphylococci*. Cases of high mortality of waterfowl at several locations of Germany were observed where *A. hydrophila* organism was isolated (Korbel and Kösters, 1989). El-Khashab (2001) experimentally infected 2 and 5 day–old chicks with *A. hydrophila* organism via yolk sac, intramuscular, subcutaneous or oral inoculations. The results revealed that some chicks died acutely while chicks that died later demonstrated a transitory period of depression characterized by ruffled feathers and pasty vent before death with mortality rate ranging 60-100%. She also observed generalized congestion of liver, spleen, lungs, kidneys, intestine (especially duodenum) with severe haemorrhagic enteritis. Moreover, there were streaks of haemorrhages on the liver's surface. Ahmed (2004) found that *A. hydrophila* induced acute death within 24 hrs of the inoculated chicks with 100% mortality rate after yolk sac inoculation and 86.6% after subcutaneous inoculation. The most predominant lesions findings were generalized venous congestion, peticheal haemorrhages on the liver, omphalitis, enteritis and nephrosis. Also, Epidemic deaths of Mallard ducks after infection with *A. hydrophila* were detected by Zbikowski *et al.* (2006).

Table (2) reveals the body weight gain of hatched chicks from GR *A. hydrophila* infected eggs and control non infected ones. The hatched chicks showed numerical difference in their weights between chicks taken from GR *A. hydrophila* infected eggs and these from non infected ones reached to 5, 30 and 103 grams at 1st, 2nd and 3rd week of age, respectively. This result explains the economic losses that may result from *A. hydrophila* infection in chickens. This finding supported those of Yadov and Verma (1998) and Kutkat *et al*. (2001) who observed retardation of growth in chicks infected with *A. hydrophila*. In addition, Ahmed (2004) detected weight gain loss in *A. hydrophila* experimentally infected chicks when compared with control birds.

From Table (3), it is observed that the rate of GR *A. hydrophila* re-isolation from dead embryos (yolk sac, liver, heart and intestine) reached 100 %. While in sacrificed survivors, the rate of re-isolation was 95.6, 26, 8.7, 4.4, 2.2 and 4.3% from intestine, liver, heart, spleen, kidney and lung, respectively. The isolation of *A. hydrophila* from the intestine of infected birds indicates intestinal colonization (Gracey *et al.* 1982). Isolation of the organism from liver, spleen, kidneys and lungs can be explained by infection via the blood stream (bacteriaemia) (Glunder and Siegmann, 1989). Recovery of GR *A. hydrophila* from extra-intestinal organs is in accordance with the findings of Shane *et al.* (1984),

El-Gohary and Amal (2002) and Zeinab *et al.* (2011). Shane and Gifford (1985) isolated *A. hydrophila* from the yolk sac, heart blood, lung, brain and cloacal swabs of experimentally infected chicks. In addition, Ocholi and Kalejaiye (1990) isolated *A. hydrophila* from liver, lung and intestine of ground hornbill suffering from haemorrhagic septicemia with haemorrhage in internal organs. Ahmed (2004) re-isolated *A. hydrophila* from heart blood and liver of experimentally infected dead chicks. Recently, *A. hydrophila* was isolated in pure culture from intestine, liver, lungs and trachea of adult ostriches died with severe necrotizing enteritis and septicaemia (França *et al.* 2009)

Considering the histopathological examination results, Figure (1) reveals that dead embryos from GR *A. hydrophila* infected eggs showed severe dilatation of hepatic blood vessels in addition to mild hemorrhages. Two-days old dead chicks hatched from GR *A. hydrophila* infected eggs showed dispersion of hepatocytes with necrobiotic changes in some of hepatic lobules and congestion of the coronary blood vessels with intramuscular oedema of the heart muscles. Figure (2) clears that twenty one day-old sacrificed chickens taken from GR *A. hydrophila* infected eggs showed pronounced oedema in addition to peripheral coagulative necrosis of the liver cells, oedema with intramuscular aggregation of inflammatory cells (mainly lymphocytes) in the cardiac muscles, hemorrhages in the intestinal villi, pulmonary oedeme with pronounced alveolar congestion and the tertori bronchioles showed subepithelial hemorrhage in the lung. These findings were similarly recorded by Ahmed (2004) and Zeinab *et al.* (2011).

To investigate the pattern of cloacal (fecal) shedding of *A. hydrophila* in infected chicken breeders, the results are investigated in Table (4). Fecal shedding of inoculated GR *A. hydrophila* revealed higher percentages in orally infected chickens (group 1 and 2) than subcutaneously infected one (group 3). However, addition of probiotic to the ration of orally infected group (group 1) resulted in lowering shedding than non-treated group (group 2). The isolation of GR *A. hydrophila* from 13% of fecal samples of raptors was reported by Needham *et al.* (1979). Moreover; Jindal *et al.* (1993) isolated motile *Aeromonads* from the droppings of 2 out 10 poultry cases. Similarly, Ahmed (2004) isolated of *A. hydrophila* from the cloacal samples of experimentally infected chicks for up to 16 days post infection. The long faecal shedding rate of *A. hydrophila* explains the serious health hazard of the pathogen especially when occur in broilers associating with an increase in the intestinal count and possible carcass contamination in poultry slaughter house. So using of probiotics for controlling of this infection is important to reduce the human health hazard.

Re-isolation of GR *A. hydrophila* from the internal egg contents (yolk) showed negative results. Results of re-isolation of GR *A. hydrophila* from outer egg shells that collected from chicken breeder groups are illustrated in Table (5). It was clarified that re-isolation of the organism from the egg shells reached 12 % in orally infected chickens whereas it reached 4 % in orally infected probiotic treated birds. No re-isolation (0%) of GR *A. hydrophila* could be determined in subcutaneously infected birds as well as in blank control ones. These results draw attention to the role of oral infection of *A. hydrophila* as a possible route of vertical transmission through intestinal colonization and contamination of egg shells during their passage via the cloaca and also spots light on the usefulness of probiotic usage in controlling vertical transmission via this route. Efuntoye (1995) and Akan and Diker (1996) identified *A. hydrophila* from different chicken' flocks when watery droppings containing mucous were examined.

No clinical signs could be noticed in GR *A. hydrophila* infected breeders via oral or subcutaneous routes. Furthermore; samples including ovary, intestine, heart, liver, spleen, kidney, lung, brain and different parts of oviduct that collected from sacrificed parent chickens gave negative results for GR *A. hydrophila* re-isolation.

In conclusion; our results are indicating that *A. hydrophila* survives for several weeks in contaminated water, ration and litter. The organism may infect birds by oral route and can colonize intestine as a part of intestinal flora. *A. hydrophila* is not congenitally transferred. It assumes a persistent nature during which shedding occurs and egg contamination takes place during the intestinal passage, therefore it seems that ovary and oviduct do not play a role in dissemination of *A. hydrophila* infection. Also, addition of probiotic to the ration can reduce fecal shedding rate as well as re-isolation of *A. hydrophila* from the egg shells.

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Table (1): The mortality rate of embryos and hatched chicks taken from GR *A. hydrophila* infected ECEs and control non infected ones

Parameter	No. of ECEs	Embryonic mortalities		Chicks mortalities during 21 days observation						Survival chicks	
				1st Week		2nd Week		3rd Week			
		No.	%	No.	%	No.	%	No.	%	No.	%
Group 1 (Infected ECEs)	60	5	8.3	8	13.3	1	1.7	0	0	46	76.7
Group 2 (Control ECEs)	25	0	0	0	0	0	0	0	0	25	100

ECEs = Embryonated chicken eggs

Necropsy findings:

- Dead embryos: Congestion of the liver, myocardium and yolk sac.
- Dead chicks: Enteritis, omphalitis, unabsorped yolk sac, distended gall bladder and congestion of liver and heart.

Table (2): The body weight gain of hatched chicks from GR *A. hydrophila* infected eggs and control non infected ones

Week / Chicks	Body weight gain (chick / gram)		
	1st Week	2nd Week	3rd Week
Group (1)	93	190	320
Group (2)	98	220	423
Difference	5	30	103

Group (1): Hatched chicks from GR *A. hydrophila* infected eggs.
Group (2): Hatched chicks from control non infected eggs.

Table (3): Re-isolation of GR *A. hydrophila* from infected 18–days old dead embryos and sacrificed survived chickens

Re-isolation from dead embryos		Re-isolation from survived chickens					
No. of dead embryos/total No. of eggs	No. of Positive cases	Intestine	Liver	Heart	Spleen	Kidney	Lung
5/60	*5/5	*44/46	*12/46	*4/46	*2/46	*1/46	*2/46
8.3 %	100 %	95.6 %	26 %	8.7 %	4.4 %	2.2 %	4.3 %

*No. of positive cases/total No. examined.

Table (4): Cloacal (fecal) shedding of GR *A. hydrophila* from chicken breeders

Days PI	Group (1)			Group (2)			Group (3)			Group (4)		
	No. of samples	+	%	No. of samples	+	%	No. of samples	+	%	No. of samples	+	%
3	8	3	37.5	8	8	100	8	2	25	8	0	0
4	8	1	12.5	8	7	87.5	8	1	12.5	8	0	0
5	8	2	25	8	8	100	8	0	0	8	0	0
6	8	0	0	8	3	37.5	8	0	0	8	0	0
7	8	1	12.5	8	6	75	8	0	0	8	0	0
8	8	0	0	8	2	25	8	0	0	8	0	0
9	8	0	0	8	1	12.5	8	0	0	8	0	0
11	8	0	0	8	2	25	8	0	0	8	0	0
13	8	0	0	8	1	12.5	8	0	0	8	0	0
15	8	0	0	8	0	0	8	0	0	8	0	0

PI= post infection += Positive %= Percentage
Group (1): Orally infected with GR *A. hydrophila* and treated with probiotic
Group (2): Orally infected with GR *A. hydrophila* and not treated with probiotic
Group (3): Subcutaneously infected with GR *A. hydrophila*
Group (4): Blank control non infected or treated

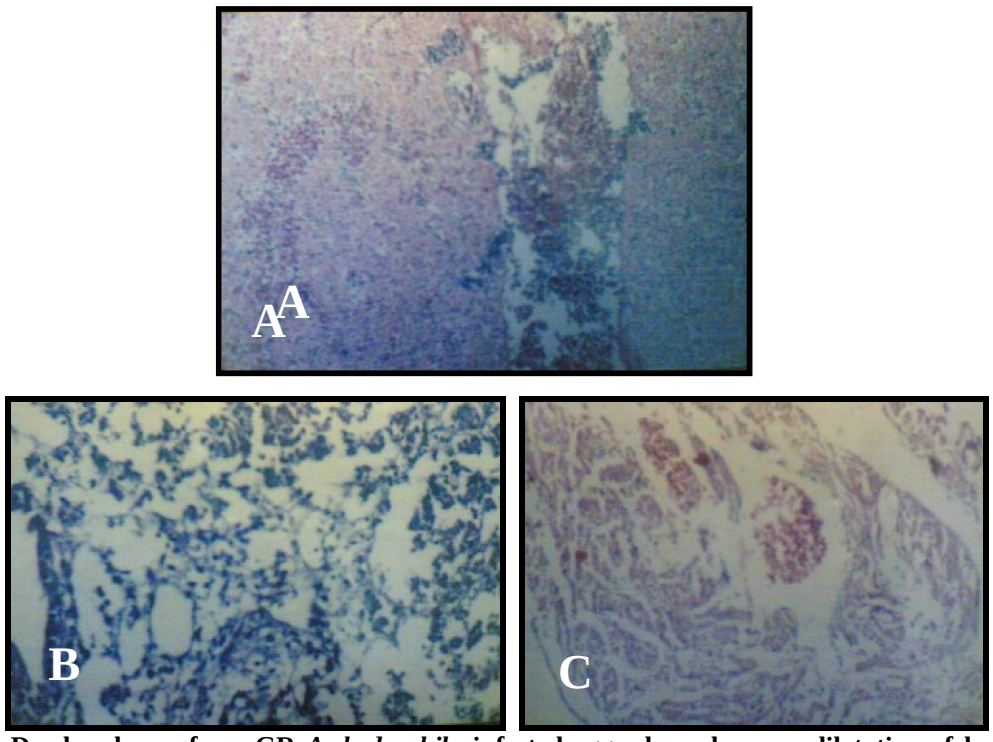

Figure (1): Dead embryos from GR *A. hydrophila* infected eggs showed severe dilatation of hepatic blood vessels in addition to mild hemorrhages (A). Two days old dead chicks hatched from GR *A. hydrophila* infected eggs showed dispersion of hepatocytes and some of hepatic lobules showed necrobiotic changes (B) and congestion of the coronary blood vessels with intramuscular oedema of the heart muscles (C).

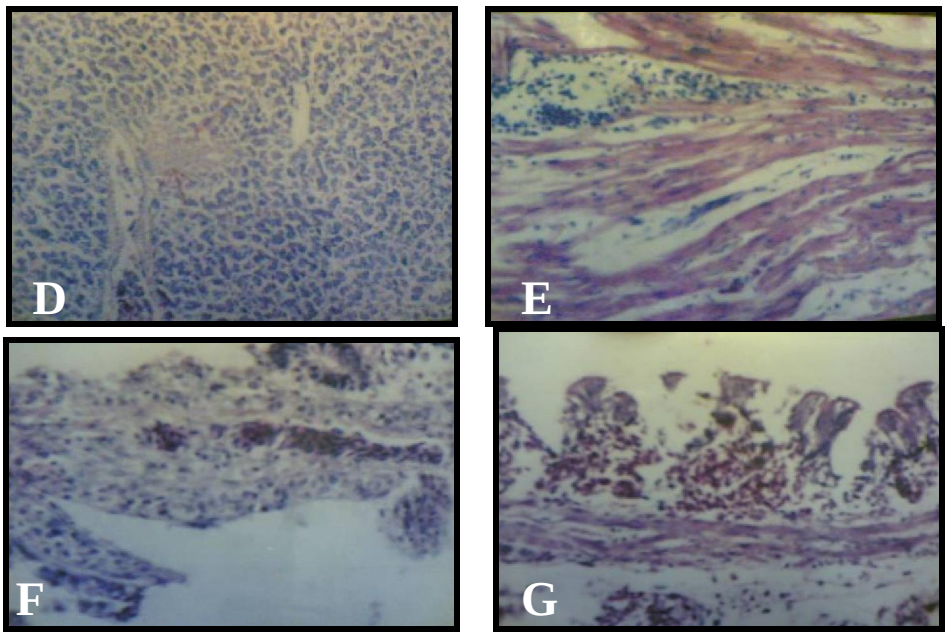

Figure (2): Twenty one day-old sacrificed chickens taken from GR *A. hydrophila* infected eggs showed pronounced oedema in addition to peripheral coagulative necrosis of the liver cells (D), oedema with intramuscular aggregation of inflammatory cells (mainly lymphocytes) in the cardiac muscles (E), hemorrhages in the intestinal villi (F) and pulmonary oedeme, pronounced alveolar congestion, and the tertori bronchioles showed subepithelial hemorrhage in the lung (G).

Table (5): Re-isolation of GR *A. hydrophila* from the outer egg shells collected from chicken breeders

No. of eggs	Group (1)		Group (2)		Group (3)		Group (4)	
	+	%	+	%	+	%	+	%
25	1	4	3	12	0	0	0	0

Group (1): Orally infected with GR *A. hydrophila* and treated with probiotic
Group (2): Orally infected with GR *A. hydrophila* and not treated with probiotic
Group (3): Subcutaneously infected with GR *A. hydrophila*
Group (4): Blank control non infected or treated

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