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.


Key Words: Aeromonas hydrophila, Chickens, Survival, Transmission

1. Introduction:


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 were added to one liter of tap water in a rate of 5×10⁹/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/liter broth, incubated at 30°C for 24 hrs; then a loopful from resultant growth was streaked onto Aeromonas 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 5×10⁹ 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 6.1×10⁹ 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 1.5×10⁹ 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 10⁹ 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 1.6x10⁹ 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 and 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 (Banchoff 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, unabsorbed 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 poult 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 Streptococc or Staphylococc. 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),
isolated motile of 10 poultry cases. Similarly, Ahmed (2004) hydrophila infection. The long faecal shedding rate of experimentally infected chicks for up to 16 days post infection. 1) resulted in lowering shedding than non-treated probiotic to the ration of orally infected group (group infected one (group 3). However, addition of probiotic to the ration can reveal higher percentages in orally infected chickens whereas it reached 4 % in orally infected probiotic treated birds. No re-isolation (0%) of 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.

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 necrotic 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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Results of re-isolation of GR A. hydrophila from the 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. Ehunttoy (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.
Table (1): The mortality rate of embryos and hatched chicks taken from GR A. hydrophila infected ECEs and control non infected ones

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of ECEs</th>
<th>Embryonic mortalities</th>
<th>Chicks mortalities during 21 days observation</th>
<th>Survival chicks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; Week</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Week</td>
</tr>
<tr>
<td>Group 1 (Infected ECEs)</td>
<td>60</td>
<td>5</td>
<td>8.3 %</td>
<td>8</td>
</tr>
<tr>
<td>Group 2 (Control ECEs)</td>
<td>25</td>
<td>0</td>
<td>0 %</td>
<td>0</td>
</tr>
</tbody>
</table>

ECEs = Embryonated chicken eggs

Necropsy findings:
- Dead embryos: Congestion of the liver, myocardium and yolk sac.
- Dead chicks: Enteritis, omphalitis, unabsorbed 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

<table>
<thead>
<tr>
<th>Week</th>
<th>Body weight gain (chick / gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; Week</td>
</tr>
<tr>
<td>Group (1)</td>
<td>93</td>
</tr>
<tr>
<td>Group (2)</td>
<td>98</td>
</tr>
<tr>
<td>Difference</td>
<td>5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Re-isolation from dead embryos</th>
<th>Re-isolation from survived chickens</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of dead embryos/total No. of eggs</td>
<td>No. of Positive cases</td>
</tr>
<tr>
<td>5/60</td>
<td>*5/5</td>
</tr>
<tr>
<td>8.3 %</td>
<td>100 %</td>
</tr>
</tbody>
</table>

*No. of positive cases/total No. examined.
Table (4): Cloacal (fecal) shedding of GR A. hydrophila from chicken breeders

<table>
<thead>
<tr>
<th>Days PI</th>
<th>Group (1)</th>
<th>Group (2)</th>
<th>Group (3)</th>
<th>Group (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples</td>
<td>+</td>
<td>%</td>
<td>No. of samples</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>3</td>
<td>37.5</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>1</td>
<td>12.5</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>2</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>1</td>
<td>12.5</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8</td>
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<td>9</td>
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<td>8</td>
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<td>13</td>
<td>8</td>
<td>0</td>
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<td>8</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>No. of eggs</th>
<th>Group (1)</th>
<th>Group (2)</th>
<th>Group (3)</th>
<th>Group (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>+ 1</td>
<td>+ 3</td>
<td>+ 0</td>
<td>+ 0</td>
</tr>
</tbody>
</table>

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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