بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وما من دآبة في الأرض ولا طائر يطير بجناحيه
إلا أمم أمثالكم
ما فرطنا في الكتاب من شيء ثم إلى ربهم يحشرون

صدق الله العظيم

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Some Epidemiological Aspects Of Aeromonas Hydrophila Infection In Poultry

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ABBREVIATIONS

A.	Aeromonas
A.hyd.	Aeromonas hydrophila.
A.A.M.	Aeromonas agar media.
C	Degree Centigrade.
CFU	Colony forming unite.
E.coli	Escherichia coli.
gm	Grams.
GR	Gentamycine resistant.
Gs	Gentamycine sulphate.
Hrs / hrs	Hours.
IV	Intravenously.
Kgs	Kilograms.
mg	Milligrams.
min	Minutes.
ml	Milliliter.
Per	for each.
Percent	percentage.
PI	Post infection
r.p.m.	Revolution per minute.
RT	Room temperature.
Um.	Micron.
Wo	Week old.

Introduction

Although the genus ***Aeromonads*** is a Gram-negative rods motile by single polar flagellum producing exotoxins, its economical significance and epidemiological importance in poultry have not been sufficiently carried out. A scanty literatures on ***Aeromonas*** species in birds are available. ***Aeromonas hydrophila*** is considered as facultative pathogen and can cause enteritis in some birds **(Aguirre et al., 1992).** ***Aeromonas*** species had been isolated in pure cultures as the primary cause of acute death, acute nephrosis, acute haemorrhagic septicaemia and conjunctivitis lesions in: canaries and toucan, a captive ground-hornbill, pet parrot, as well as captive bustards **(Panigraphy et al., 1981; Ocholi and Kalejaiye,1990; Garcia et al., 1992; Silvanose et al., 2001) .**

In domestic poultry, ***Aeromonas hydrophila*** had been isolated from septicaemic condition of 3-16 weeks-old commercial turkeys, poultry faeces, haemorrhagic septicaemia in ducks, from faeces and carcasses of broiler chickens as well as from out breaks in duck folks suffered from sudden death with clinical signs of anorexia and dyspnoea **(Gerlach and Bitzer 1971; Stern et al., 1987; Jindal et al., 1993; FanDe et al., 1997; Akan et al., 1998; and KeMin et al., 1998).**

Aeromonas species are considered as food born pathogens and of public health importance **(Garcey et al., 1982; Agger et al., 1985; Hardy et al., 1986; KiRov et al., 1990 and Sarinehmetoglu and Kuplulu,2001).**

Burke et al., (1984) and KiRov et al., (1990) discussed the epidemiology of ***Aeromonas hydrophila*** through food contamination, infected water, food contaminated with animal faeces and infected handlers. ***Aeromonas hydrophila*** can contaminate treated water (chlorinated) and chill water used in poultry industry.

Khurana and Kumar (1997) could isolate 125 isolates of ***Aeromonas*** species from poultry eggs, liver, heart and meat in ratio of 0.9%, 32.6% and 28% respectively, where ***Aeromonas hydrophila*** was the predominant isolate (77 out of 125 isolates).

The present investigation was designed to investigate:

1-The in–ovo infection with ***Aeromonas hydrophila***.
2-The survival time of ***Aeromonas hydrophila*** in tap water and different materials under controlled laboratory conditions.
3-The possibility of transovarian transmission of ***Aeromonas hydrophila*** in parent chickens.

REVIEW OF LITERATURE

Taxonomy:

Kluvyer and van Neil (1936) proposed that genus ***Aeromonas*** be used to encompass microorganisms that are phenotypically similar to the enteric group but motile by means of a polar flagellum.

Stanier (1943*)* noted that many organisms previously described as members of the genera ***pseudomonas***, ***peoteus, Bacillus*** and ***Aerobacter*** were really members of the genus ***Aeromonas***.

Snieszko (1957) differentiated three species of motile ***Aeromonads*** based upon pathogenicity and biochemical tests: ***A. hydrophila, A. punctata and A. liquefaciens***.

Page (1962) and Schubert (1967) reiterated the existence of sufficient biochemical similarities to separate the genus name but they were not concur as to how the motile ***aeromonads*** should be divided into species.

Popoff and veron (1976) in a computerized numerical taxonomy study, examined 68 strains of ***Aeromonas*** for 203 morphological, biochemical and genetic properties. They suggested that the group of organisms belonging to ***A. hydrophila*-A. punctata** taxons be classified as ***A. hydrophila*** and a new species**,** ***A. Sobria*.**

Maclnnes et al., (1979) examined polynucleotide sequences among 24 motile ***Aeromonads*** and found that these organisms, variously described phenotypically as ***A. hydrophila. A. punctata*** and ***A. Sobria*** did not possess internal homology groups which were significant divergence in related sequences.

Popoff et al., (1981) divided motile aeromonads into three species: ***A. hydrophila, A. Caviae*** and ***A. sobria*** on the basis of phenotypic and genetic evidence.

Baumann and Schubert (1984) in Bergey's Manual of systematic Bacteriology listed ***Aeromonas*** species along with ***Vibrio*** species and ***plesiomonas shigelloides*** in the family ***Vibrionaceae*** on the basis of these characteristics. They classified ***Aeromonas*** species into four species: ***A. hydrophila , A. Caviae, A. sobria*** and ***A. salmonicida***. **Popoff (1984)** mentioned that members of genus ***Aeromonas*** are clearly differentiated from members of the ***Enterobacteriaceae*** and members of the genera ***pseudomonas*** and ***Vibrio***.

Colwell et al., (1986) recommended the removal of Aeromonas species from ***Vibrionaceae*** and placing of them in a separate family, ***Aeoromonadaceae.***

Koneman et al .,(1992) recognized 12 DNA hybridization groups (also called genospecies) within the genus ***Aeromonas***. They mentioned that some of the DNA hybridization groups can not be phenotypically separated. Therefore; some of the groups were not named uptill this date.They also grouped genus ***Aeromonas*** into two subdivitions as follows :

1-***Psychrophilic group***: containing ***A. salmonicida*** as the only species in this group and is non motile fish pathogen which can not grow at 37^{o}C.

2- ***Mesophilic group***: containing ***A.hydrophila*** members which can grow at 37^{o}C . and are motile. ***Mesophilic group*** can be divided into three principal phenotypic groups which are equivalent to the species ***A. hydrophila, A. caviae*** and ***A. Sobria.***

Altwegg (1999) stated that genus ***Aeromonas*** includes four phenotypical separable species ***A. hydrophila A. caviae, A. Sobria*** and ***A. salmonicida***. He also added that several new phenotypic species and 16 DNA hybridization groups (HGs) have been described, some of which were not yet named. ***A. hydrophila*** phenospecies contain three DNA HGs: 1, 2 and 3. HG1 has been named ***A. hydrophila*** genospecies. HG 2 has been named ***A. bestiarum*** genospecies and HG3 which was unnamed

Morphplogical , cultural , biochemical , and enzymatic characteristics of A. hydrophila :

Shotts and Rimler (1973) designed a differential medium for isolation of motile aeromonads. ***A. hydrophila*** colonies appeared after incubation at 37^{o}C for 24- 48 hours as yellow rounded colonies. This medium was designed to facilitate diagnosis of ***A. hydrophila*** infections.

Schubert (1974) described the original definition of ***A. hydrophila*** to be facultative anaerobe, Gram negative, straight rods, either motile by means of a polar flagellum (generally monotrichous) or non-motile. He also added that ***A. hydrophila*** strains are carbohydrate fermenters with production of acid or acid and gas. They are oxidase positive, reduce nitrates to nitrites and insensitive to the vibriostatic compound (0/129) and a guanine cytosine content (G-C ratio) of 57-63%.

Popoff and Veron (1976) reported that ***A. hydrophila*** is Gram negative straight rods measuring approximately 0.5 X 1.0 micron, carbohydrate fermenters with the formation of acid or acid and gas; cytochrome oxidase positive, reduced nitrates and insensitive to the 0/129 compound.

Buxton and Fraser (1977) described ***A. hydrophila*** as follows: rod-shaped organisms and most strains have a polar flagellum, motile Gram–negative, ferments a variety of carbohydrates including glucose with production of acid and gas but some strains produce acid only. Fermentation of lactose is variable. The organism produces indole and H2S but not urease. The methyl red test is positive while the Voges- proskauer is variable.

Lennette et al., (1980) described that members of genus ***Aeromonas*** are Gram–negative rods, possessing polar, usually monotrichous flagella. ***Aeromonads*** produce catalase, oxidase and ferment glucose and other carbohydrates with production of acid or acid and gas. Nitrate is reduced to nitrite.The optimal growth temperature is 30^{o}C; many strains are biochemically more active at 22 ^{o}C than at 37 ^{o}C.

Waltman et al., (1982) used the APL ZYM system to determine the enzymatic characterization of 48 ***A. hydrophila*** isolates from various sources. **Popoff (1982)** mentioned that strains of ***A. hydrophila*** could be maintained on trypticase soy agar, after incubation to allow good growth, the cultures were kept in refrigerator (4-8 ^{o}C) for at least one month. They could also preserved by freeze-drying.

Palumbo et al., (1985) studied the growth of clinical isolates of ***A. hydrophila*** at various temperatures, pH values and salt levels in brain heart infusion (BHI) broth. They noticed that most isolates were better to tolerate 4% NaCl and acidic pH when cultured at 28 ^{o}C as compared to 4 ^{o}C.

Figura et al., (1986) used the AP1 20 E system for further identification of ***Aeromonas***. AP1 20 E strips were incubated at 37 ^{o}C. Most of the results were recorded after 24 hours of incubation; the V.P. and lysine decarboxylase reactions were read after additional 2 days of incubation at room temperature. **Finegold and**

Baron (1986) stated that the adaptation of macrobroth dilytion antimicrobial susceptibility test to a microdilution format. This test utilized plastic microdilution trays and was used for routine reporting of MIC results.

Namdari and Bottone (1989) studied the suicide phenomenon as a simple mean of differentiating between ***A. caviae, A. hydrophila*** and ***A. sobria*** by growth them in broth media containing 0.5% glucose. They found that ***A. hydrophila*** isolates were nonsuicidal, aerogenic and esculin positive.

Vadivelu et al., (1991) mentioned that ***A. hydrophila*** a member of the family ***Vibrionaceae*** is increasingly recognized as a pathogen of man which causing both intestinal and extraintestinal infections. **Varnam (1991)** proposed a number of enrichment broth for ***Aeromonas*** including tryptone- soya broth, tryptone-soya broth plus ampicillin, tryptone-soya broth plus Nacl, and tryptone broth. He has also pointed out that ***A. hydrophila*** can be isolated on many of selective media devised for members of Enterobacreacae such as MacConkey agar, which has been modified by addition of ampicillin, Salmonella- Shigella agar and cefsulodin- irgasan –novobiocin (CIN) agar .

Koneman et al., (1992) mentioned that ***A. hydrophila*** is a motile mesophilic bacterium which can grow at 37 ^{o}C. They also isolated the organism on 5% sheep blood agar containing 10mg/L ampicillin and cefsulodin–irgasan–novobiocon (CIN) media They also added that ***A. hydrophila*** is oxidase positive and can be quickly excluded from ***Enterobacteriaceae*** by performing an oxidase test and can be differentiated from ***Pseudomonas*** species by glucose fermentation test.

Hanninen (1994) confirmed that the maximum growth temperature of ***A. hydrophila*** HG1 strains was significantly higher (41±0.56^{o}C) than that of HG2 (38±0.69^{o}C) or HG3 (38.6±0.75^{o}C) He has also suggested the following biochemical tests in order to separate ***A. hydrophila*** at the hybridization group level ; utilization of citrate and fermentation of D-rhamnose, D-sorbitol and DL-lactose. **Quinn et al., (1994)** described the colonial morphology of ***A. hydrophila*** on blood and MacConkey agars. Colonies were small, beta hemolytic on blood agar and pale in color on MacConkey agar (mainly non lactose fermenter but minority of strains yield lactose fermenting colonies).plates were incubated at 37^{o}C for 18-24 hours. They also found that ***A. hydrophila*** is catalase, gelatin and nitrate reduction tests positive.

Collee et al., (1996) mentioned different biochemical tests used for differentiation and identification of most of bacterial populatins. Oxidase, catalase, urease, sugar fermentation and other tests were clearly described.

Atlas and parks (1997) high lighted that several media were developed for primary isolation of ***A. hydrophila*** as ***Aeromonas*** medium, ***A. hydrophila*** medium, pril xylose ampicillin agar (PXA), Fay and Barry medium and Rippey-cabelli agar (RC) .They reported that ***Aeromonas*** medium can be used for isolation and selective differentiation of ***A. hydrophila*** and other Aeromonas species from clinical and non clinical samples. They also detected that ***Aeromonas*** species appear as small (0.5-1.5 mm), green colonies with darker centers on ***Aeromonas*** medium.

Atwegg (1999) described ***A. hydrophila*** as Gram negative straight, rod shapped to coccoid cells with rounded ends measured 1.0-3.5 µm long and 0.3-1.0 µm wide with polar, usually has monotrichous flagellum but peritrichous flagella may be formed in young cultures on solid media. He mentioned that ***A. hydrophila*** is facultative anaerobe, oxidase and catalase positive and ferment D-glucose to acid and gas. He also added that ***A. hydrophila*** is positive indole, lysine decarboxylase, Voges-Preskauer and produce acid from mannitol, arabinose and salicin sugar. The organism is negative ornithine decarboxylase, lactose fermentation tests and is resistant to vibriostatic agent 0/129 and ampicillin. He also pointed out that many exoenzymes are produced as DNAse, urease, estrases, peptidases and other hydrolytic enzymes. The optimal growth temperature was between 22-28^{o}C but growth also occurred at 37^{o}C. He also noticed that a presumptive identification of ***A. hydrophila*** can be based on positive oxidase test, growth on MacConkey agar fermentation of D-glucose to acid and gas.

Sachan and Agarwal (2000) tested six selective agents (ampicillin, novobiocin, cefalotin, bile salts, brilliant green and ethanol) during the development of a selective enrichment broth for isolation of ***Aermonas*** species from chicken meat. Cefalotin at 10 mg / L was found to be the most selective agent.

Epidemiological Aspects :

Rippey and Cabelli (1980) studied the densities of ***A. hydrophila*** in various natural water of lrland. They reported that the bacteria seemed to be seasonally distributed with maximum count during summer through early fall .

Kaper et al., (1981) investigated the density and standard bacterial count of ***A. hydrophila*** during winter season .

Shane and Gifford (1985) found that 2 to 4 day-old experimentally infected chicks were highly susceptible to ***A. hydrophila*** exposure via s/c, yolk sac and intracerebral routs with mortality rate of 80-100%. They also isolated the organism from yolk sac , heart blood , lung , brain and cloacal swabs of experimentally infected chicks .

Arcos et al., (1988) isolated ***A.hydrophila*** from polluted water. They also evaluated several selective media for the recovery of the organism .

Araujo et al., (1991) recorded that ***A. hydrophila*** was the most abundant species isolated from water with low faecal pollution between the presence of ***Aeromonas*** species and degree of water pollution. **Poffe and Op de Beeck (1991)** examined quantitatively influents, effluents and sludges from sewages purification plants and surface water samples for ***A. hydrophila*** on Rippey and Cabelli medium. They concluded that ***A. hydrophila*** was omnipresent in surface water. **Varnam (1991)** reported that ***A. hydrophila*** was isolated from chlorinated and unchlorinated piped water supplies. The organism has been isolated from bored well water.

Jindal et al. (1993) isolated motile aeromonads from faeces of poultry. They isolated motile ***aeromonads*** from faeces of 2 of 10 poultry cases where sensitivity test was discussed.

Quinn et al. (1994) proposed that ***Aeromonas*** species are wide spread in fresh water, sewage and soil. They also noticed that the numbers increased with the amount of organic matter present.

Efuntoye (1995) identified ***A. hydrophila*** when watery faeces containing mucous from fowls were examined during a farm out break of diarrhea. **Vadivelu et al. (1995)** suggested that the range of human extra intestinal infections is from traumatic introduction of that ***A. hydrophila*** microorganisms into the skin.

Akan and Diker (1996) isolated ***Aeromonas*** species from chicken faeces when faecal samples of different poultry flocks were examined. They added that ***A. hydrophila*** was the most prevalent in these samples. **Rosskopf and Woerpel (1996)** found that birds are usually exposed to infection with ***A. hydrophila*** through their food and transmission is primarily by oral routes with faecal shedding into environment.

Akan et al., (1998) isolated ***A. hydrophila*** from chickens faeces, carcasses, scalding water and chilling water during slaughtering process in commercial processing plant. They detected that, during slaughtering process, the spread of motile ***aeromonads*** from the intestinal contents to the carcasses via processing water caused a heavy contamination of chicken carcasses. **Fiorentini et al., (1998)** isolated a total of 208 strains of ***Aeromonas*** by monthly sampling from estuaries (one provided with, and the other devoid of a waste-water treatment system) on the Italian coast of Adriatic Sea between September 1994 and August 1995. Biotyping at the species level allowed the identification of 96 strains (46%) as ***A.caviae,*** 46 strains (22%) ***A.sobria***, 33 strains (16 %) as ***A. hydrophila*** and 25 strains (12%) as ***A.veronii***. Eight strains (4%) were regarded as unnamed ***aeromonads***. **Kelley et al., (1998)** isolated ***A. hydrophila*** and other bacteria for during the microbial evaluation of coarse fraction of litter for its reutilization as a bedding supplement in growing flocks of broilers. They also tested these bacterial isolates for their sensitivity to 12 common antibiotics. **Legnani et al., (1998)** studied the occurrence of ***Aeromonas*** species in drinking water supplies in a mountain area in northeast Italy (the Dolomites). Most of the isolates were identified as ***A. hydrophila*** and it was believed that the search for these micro-organisms should be adopted as a further indicator of drinking water quality.

Altwegg (1999) stated that ***Aeromonads*** are mainly found in aquatic environment such drinking water. Ground water, clean water, Lakes, Waste water, treated sewage, crude and domestic sewage sludge. They also found that ***Aeromonads*** may be occasionally present in marine environments, although these organisms seem to prefare salt waters interfacing with fresh water.

EL-Khashab (2001) in Egypt experimentally infected 2 and 5 day–old chicks via routes of yolk sac, I/M, S/C and orally. The results revealed that chicks were highly susceptible to the infection with the local isolates of ***A. hydrophila*** with mortality rate ranged from 60-100% . **Sarimehmetoglu and Kuplulu (2001)**

analyzed a total of 140 broiler carcasses parts, purchased at different supermarkets in Ankara, for the presence of motile ***Aeromonas*** species. Motile ***Aeromonas*** species were isolated from 116 (82.9%) of the 140 samples. ***A . hydrophila*** was the most prevalent species isolated (56%). Followed by ***A. sobria*** (29.3%) and ***A. caviae*** (14.7%) from all the carcasses and carcass parts, respectively. Consequently; it was supposed that examined samples were contaminated to significant levels with motile ***Aeromonas*** species and posed as a public health risk.

Glunder (2002) studied the influence of diet on the occurrence of some bacteria in the intestinal flora of wild and pet birds. He examined ***A. hydrophila*** in nearly 3500 wild and pet birds and found that infection was found in 1.9% of the granivorous and herbivorous species, in 7.1% of the omnivorous and in 12.4% of the carnivorous and insectivorous birds. He concluded that the occurrence of ***A. hydrophila*** in the digestive tract of birds is obviously influenced by the composition of the nutrients.

Pathogenicity and virulence factors :

Duby and Sanyal (1979) characterized and neutralized ***A. hydrophila*** enterotoxins using rabbit–ilial-loop model . They used six strains of ***A. hydrophila*** isolated from different sources including patients suffered from diarrhea. Calves and water of a hand pump. **Nicholas et al., (1979)** studied the effect of cytotoxins and enterotoxins produced by ***A. hydrophila*** in Hela cells (tissue culture) they found that after 24 hours exposure to ***A. hydrophila*** culture filtrate the cells has pycnotic nuclei, lost their adherence properties and became round and shrunken. They suggested that rounding of cells may be due to proteolytic substances produced by ***Aeromonas*** species.

Monica and Asa (1981) studied the effect of two types of haemolysins produced by ***A. hydrophila*** on human fibroblasts . They indicated there were morphological changes of lung fibroblasts with alpha and beta–haemolysins. The exposure of the cells to alpha–haemolysin lead to rounding and retraction of the cells from each other and the cytoplasm was finely granular while the nuclei were not clearly visible the exposure to beta-haemolysin induced characteristic vacuolization of the cytoplasm while the nuclei and nucleoli were distinctly seen.

Jiwa (1983) found that about 90% of ***A. hydrophila*** isolates agglutinated bovine, chicken, human group A and guinea-pig erothrocytes in the presence of mannose at $4^{o}C$ and/or $20^{o}C$.

Shane et al., (1984) recorded that ***A. hydrophila*** is a facultative pathogen that occurs infrequently but may be significant in individual birds debilitated by environmental stress, inter current infection or injury.

Figura et al., (1989) isolated ***A. hydrophila*** from human faecal samples with gastroenteritis and from controls. They found no significant difference between the prevalence of enterotoxin-producing strains (suckling mouse model),

cytotoxin-producing strains (Hep-2 cell model) or haemolysin- producing strains (rabbit erythrocyte model) between patients and controls.

Rita and Janda (1987) *evaluated* 58 isolates of motile ***Aeromonads*** for the ability to produce B- haemolysin. They found that haemolytic activity was significantly associated with strains belonging to the ***A. hydrophila*** and ***A. sobria***.

Glunder (1988) isolated ***A. hydrophila*** from 80 birds from atotal 2236 perished birds. He found that mono infection was found in 4 cases while in all other cases the ***A. hydrophila*** infection was combined with the presence of ***Enterobacteriaceae*** and/or streptococci or staphylococci. The author noticed that predisposing factors seem to be necessary to provoke the outbreak of the disease.

Marian (1989) demonstrated that the main virulence factors of motile aeromonads caused by ***A. hydrophila*** are as follows: Enterotoxina (Cytotonic enterotoxins and Cytotoxic enterotoxins), haemolysins, proteases, haemagglutinins, endotoxins, ability to adhere to cells and possession of certain surface proteins. She reviewed the mode of action of each virulence factor that contributes to their pathogenicity. **Palumbo et al., (1989)** studied virulence factors of ***A. hydrophila.*** They found that most food and clinical isolates were serum resistant, beta-haemolytic and cytotoxin positive.

Janda (1991) reviewed that the virulence factors of ***Aeromonas*** species can be divided into three categories: a)–Structural features (pili, surface layer, lipopolysaccharide (L.P.S.) and outer membrane proteins (OMPs). b)-Extracellular factors (haemolysin, proteases and siderophores). c) –Cell-associated factors (invasions, serum resistance, plasmids and adherence). **Varnam (1991)** recorded the pathogenicity and virulence factors of ***Aeromonas*** species and mentioned that virulence factors of ***Aeromonas*** species can be summarized as follow: 1-Adherence or presence of Pili. 2-The presence of surface layer (S-layer) (which was involved in systemic infections especially in immunocompromised hosts), 3-Toxins: a)-enterotoxins (cytotonic enterotoxin, cytotoxic enterotoxin, cytolytic enterotoxin), b)-proteases, c)-sodium channel inhibitor. These toxins have direct causes of enteritis and facilitates establishment of the organism in the intestine and aid adherence and for invasion. He also described a number of phenotypic characters which have been proposed as markers of enteropathogenicity such as haemolysis, lysine decarboxylase activity, Voges-Proskauer reaction and sorbitol fermentation.

Vadivelu et al., (1995) isolated eighteen strains of ***A. hydrophila*** from patients with bacteraemia and investigated these strains for the possible virulence factors and found that all strains were producing cytotoxin and haemolysin .

Rosskopf and Woerpel (1996) concluded that ***A. hydrophila*** appears to be a facultative pathogen for birds, requiring the host to be compromised before disease occurs as stress, high environmental temperatures, concurrent bacterial infections (especially ***Salmonellae***) and injury to assist the disease progress .

Barnes (1997) recorded that ***A. hydrophila*** either alone or incombination with other pathogens , can cause localized or systemic infections in avain species .

Clinical and Pathological Picture of A. hydrophila infection in Birds:

Gerlash and Bitzer (1971) described a septicemic condition of commercial turkeys aged 3-16 weeks in Germany that was attributed to ***A .hydrophila***. Flock morbidity ranged from 10%-30% and mortality of 1%-5%.ded in a series of cases submitted for diagnosis.

Panigraphy et al., (1981) described cases of ***A. hydrophila*** infection in canaries, a toucan and a cockatiel. They isolated ***A. hydrophila*** in pure culture from a toucan (Ramphastos toco) with acute nephrosis and a cockatiel (Nymphicus hollandicus) with ***Chlamydiosis*** (psittacosis).

Shane and Gifford (1985) reported that experimentally infected chicks with ***A. hydrophila*** either acutely died without showing premonitory signs or after a transitory period of depression which characterized by ruffled feathers. They also found that there were no specific lesions except an evident generalized venous congestion including focal cerebral plaques and petechial hemorrhage on the mucosa of the proventriculus and jejunum. Pulmonary congestion and hepatic petechiae were also recorded. Histopathological lesions comprised severe multifocl acute coagulative necrosis of the neuropil, congestion of liver and extensive pulmonary necrosis.

Garcia et al., (1992) recorded bilateral conjunctivitis in a pet parrot (Amazona versicolor) . They isolated ***A. hydrophila*** in pure culture from both eyes.

Bisgaard (1995) studied the prevalence of salpingitis in web-footed birds. He recorded 6 cases out of 1548 caused by ***A. hydrophila*** infection.

FanDe et a.l, (1997) reported on an outbreak of red leg disease in frogs and of haemorrhagic septicaemia in ducks farm in fujian, China. Liver and kidney specimens were sampled from affected and dead bullfrogs and ducks for the isolation of pathogenic organisms. Two isolates of ***A. hydrophila*** were recovered.

Olkowski et al., (1999) isolated ***A. hydrophila*** from several cases of cellulitis in turkeys. They stated that birds showed no obvious clinical signs but some affected birds were emaciated, cyanotic or showed signs of air sacculitis and peritonitis.

EL-Khashab (2001) in Egypt experimentally infected chicks and observed that some chicks died acutely while chicks that died late demonstrated a transitory period of depression characterized by ruffled feathers and pasty vent before death. She also observed a generalized S/C venous congestion as well as congestion of liver, spleen, lungs, kidneys, intestine especially duodenum showed severe haemorrhagic enteritis , liver also have streaks of haemorrhage in experimentally infected chicks .

Antibiograming and control of A . hydrophila infections:

Varnam (1991) reviewed that antimicrobial therapy is indicated for treatment of ***A. hydrophila*** infections. The drugs of choice were chloramphenicol,

tetracycline and cotrimoxazole. He also added that other antibiotics may be used but the in vitro sensitivity testing appears to be very important for the use of accurate antimicrobial drug.

Barnhart and pancorbo (1992) determined the antibiotic resistance profiles of ***A. hydrophila*** isolates recovered from broiler carcasses and chill water samples taken from a Georgia processing plant. They reported that all isolates were resistant to ampicillin (greater than 90%), multiple antibiotic resistance occurred, most of them were resistant only to ampicillin and cephalothin . **Garcia et al., (1992)** studied the biochemical characteristics and antimicrobial susceptibility of ***A. hydrophila*** strain isolated from a case of conjunctivitis in a pet parrot. Antimicrobial susceptibility assayed by an agar disc diffusion method showed that ***A. hydrophila*** isolate was susceptible to chloramphenicol, tetracycline and cefoxitin and resistant to ampicillin, penicillin G and cephalothin.

Russel et al., (1993) reported that the lactic acid fermentation appears to be effective in reducing the number of ***A. hydrophila,*** other bacterial pathogens and indicator organisms in poultry processing offal.

FanDe et al., (1997) tested the susceptibility of two isolates of ***A. hydrophila***. They found that they were sensitive to gentamicin and neomycin. **San et al., (1997)** tested the susceptibility of ***A. hydrophila*** isolates to 10 antimicrobial agents. Of the 21 fish isolates examined, all were resistant to ampicillin and sensitive to gentamicin. Most isolates were resistant to streptomycin (57%), tetracycline (48%) and erythromycin (43%) .

Forbes et al., (1998) found that members of genus ***Aeromonas*** are capable of producing various beta-lactamases that mediate resistance to penicillin and certain cephalosporins. **Kelley et al., (1998)** reported that ***A. hydrophila*** isolates were resistant to ampicillin, penicillin, tetracycline and streptomycin and were susceptible to erythromycin, gentamycin , kanamycin, nalidixic acid, neomycin and sulfasoxazole. They also added that the use of subtherapeutic doses of antibiotics as feed supplements may hamper attempts to re-utilize litter and should be re-examined in light of the possible selection of antibiotic-resistant microbial populations in the litter.

Altwegg (1999) reviewed that most ***Aeromonas*** species are resistant to penicillin , ampicillin , caebenicillin and ticarcillin and susceptible to expanded and broad spectrum cephalosporins. Aminoglcosides,carbapenems, hloramphenicol, tetracyclines, trimethprim – sulfamethoxazole and quinolones .

Benassi et al., (2001) investigated the susceptibility of ***Aeromonas*** species, isolated from chicken carcasses to beta lactam antibiotics. All tested microorganisms were susceptible to third generation cephalosporin, cefepime, imipenem. Aztreonam and were resistant to ampicillin. **EL- Khashab and El – Yased (2001)** in Egypt studied the in-vitro sensitivity test of ***A. hydrophila*** isolated from experimentally infected ducks. They found that it was highly sensitive for kanamycine, streptomycin, tetracycline, Lincospectin, neomycin and oxytetracycline; moderately sensitive to ampicillin, chloramphenicol,

amoxycilline and nalidixic acid and weakly sensitive to erythromycin and nitrofurantion.

Materials and Methods

1-Aeromonas hydrophila strain:

A strain of ***Aeromonas hydrophila*** has been originally isolated from poultry meat meals were used in this study. These meals were submitted to Animal Health Research Institute, from abroad for routine examination of salmonellae species. Isolation of ***A. hydrophila*** has been carried out in trypticase soy broth containing 10 mg / ml ampicillin which was inoculated by 1gm of sample and incubated at 28 – 30^{o}C for 24 hrs. A loopful from the incubated broth was streaked onto trypticase soy ampicillin agar (TSA) and MacConkey agar plate and incubated at 28 – 30 ^{o}C for 48 hrs. Suspected colonies were picked up for further identification. The isolated organism was identified biochemically according to **Popoff and Veron (1976)** employing Gram stain, motility test, Vogoes-Proskauer test, indole production, gelatin liquefication, suger fermentations, oxidation test and aesculin broth hydrolysis.

2-Embryonated Chicken Eggs (ECE):

A total of 120 Hubbard ECE obtained from a Commercial poultry Hatchery (AL-AHRAM CO.) were used.

3-Experimental chickens:

Thirty broiler breeder hens (33 weeks old) and four cocks (29 weeks old) were used in this study. They were vaccinated against NDV and AI.

3-Probiotic:

A natural probiotic premix of selected lactic acid bacteria **containing** 10^{9} CFU/g. of ***Pediococcus acidi lactic*** produced by Lallemand Co.; France under the trade name ***Bactocell*** in a dose of 1 Kg/Ton feed, Batch No. 402060 was used.

4-Culture Media:

Media for primary isolation and purification of bacteria:

****Aeromonas*** agar (Oxoid; composition (g/L):

Proteose peptone	5.0
Yeast extract	3.0
L.lysine monohydrochloride	3.5
L.arginine monohydrochloride	2.0
Inositol	2.5

Lactose	1.5
Sorbitol	3.0
Xylose	3.75
Bile salts no.3	3.0
Sodium thiosulphate	10.67
NaCl	5.0
Ferric ammonium eitate	0.8
Bromothymol blue	0.04
Thymol blue	0.04
Agar	12.5

29.5 g were suspended in 500 ml of distilled water, boiled then left to cool to 50^{o}C before mixing and pouring into plates.

*MacConkey agar (Oxoid)

*Brain heart agar media (Oxoid)

5-*Media for biochemical identification of bacteria*:

***Sugar fermentation media:**
Using 1% peptone water containing 1% bromocresol purple indicator to which 1% of each of the following sugars was added: mannitol, glucose, lactose, salicin and L-arabinose.

***Urea agar base (Oxoid):**
This medium was used in urea hydrolysis test.

***Semi – solid 0.5% (soft) agar:**
This medium was used for the detection of motility as well as for the preservation of all isolates during the course of present study.

***Nutrient agar (Oxoid).**

*** Soft agar media for preservation.**

6-Reagents:

1.Cytochrome Oxidase (N N-dimethy 1-P –phenylene diamine dihydrochloride(BDH).
2.Bromocresol purple.
3.Hydrogen peroxide (10 volumes).
4.NaCl.
5.Gentamicin sulphate (Garamycin) (Schering plough).

7-Stains and Buffers :

1. Gram's stain
2. Normal saline 0.9%.

8-Preparation of Aeromonas hydrophila gentamicin resistant strain

Aeromonas hydrophila was preserved on soft agar media after biochemical identification, the strain was cultured on nutrient broth media incubated at 37^{o}C for 24 hours under sterile conditions the growth was streaked onto ***Aeromonas*** agar media and MacConkey agar media and incubated at 37^{o}C for 24 hours, this step repeated three times for the refreshment of the microorganism.

Aeromonas hydrophila was rendered gentamycin resistant using the method of **Reid et al. (19610 and Awaad (1975)** as follows: The organism was subcultured in successive broth cultures containing increasing quantities of gentamycin, starting with 2 µg/L and ending by 100 mg/L broth, then subculcutured on ***Aeromonas*** gentamycin agar plates (***Aeromonas*** agar containing 100 mg gentamycin per litre). This ***Aeromonas hydrophila*** gentamycin resistant strain will be reffered to as GR ***Aeromonas hydrophila.***

9-Biochemical Examinations:

These were adopted after **Crwickshank et al. (1970)** using oxidase, catalase, indole, citrate, urease, Voges-Proskauer, cytochrome oxidase tests, H_2S production as well as sugar fermentation of mannitol, lactose and sorbitol.

Experiments and Results

Experiment 1

Study on the possible transmission of *Aeromonas hydrophila* via egg shell penetration (the in-ovo infection) :

Eighty five, 18 day-old embryonated chicken eggs (ECE) were used in this experiment. They were divided into 2 groups (1 and 2) consisting of 60 and 25 ECE respectively. ECE of group one were infected with ***GR Aeromonas hydrophila*** by dipping in 18 hours chilled broth culture containing 6.1×10^{9} viable bacterial cells (CFU/ ml) for five minutes. ECE of group 2 were similarly dipped in sterile nutrient broth as a control group. ECE of both groups were further incubated with daily observation for embryo livability and mortality. Hatched chicks were kept under observation for 21 days for clinical signs and mortality. Dead ECE as well as dead and sacrificed survived chicks at the end of observation period were necropsied and subjected for bacteriological examination in attempt of ***GR Aeromonas hydrophila*** reisolation. Specimens were collected from necropsy birds for histopathological examination.

Results:

Results are shown in tables 1-3 and figs.1-4.

Distended gall bladder and septicaemic picture were noticed in dead in shell embryos. Dead chicks were suffering from enteritis, omphalitis, unabsorbed yolk sac, distended gall bladder, congestion of liver,heart and septicaemic picture.

Fig.1: Colonial morphology of ***Aeromonas hydrophila*** on MacConkey agar media

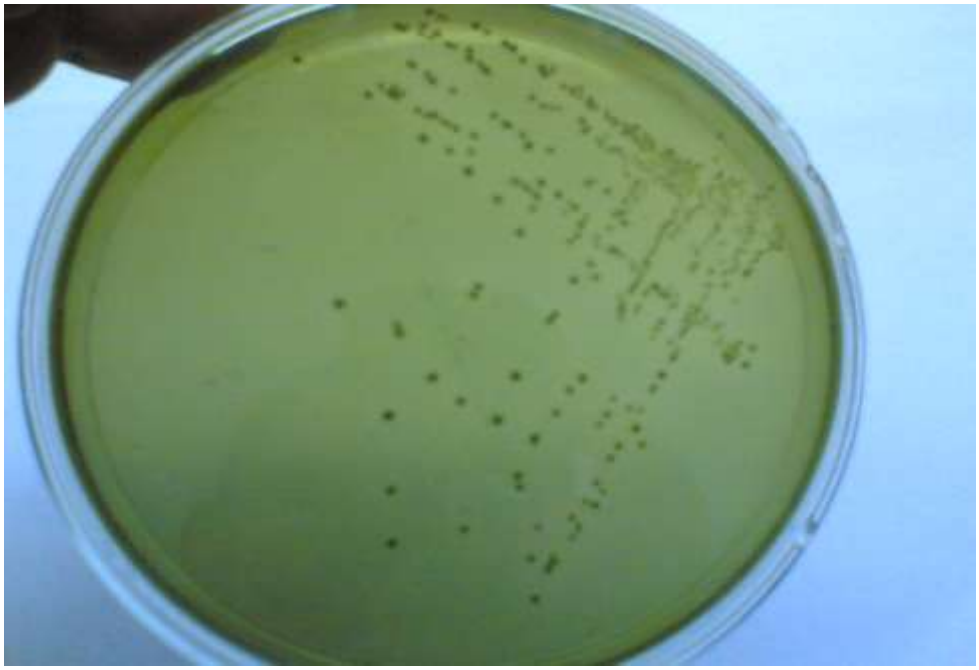

Fig.2: Colonial morphology of ***Aeromonas hydrophila*** in selective ***Aeromonas*** agar media

Table (1): Different parameters in assessing the in–ovo infection with *GR Aeromonas hydrophila*

Parameter	No. Of ECE	Embryonic mortality		Chicks mortality during 21 days observation						Survival chicks	
				1ST Week		2nd Week		3rd Week			
		No.	%	No.	%	No.	%	No.	%	No.	%
Group 1 (Infected ECE)	60	5	8.3	8	13.3	1	1.7	0	0%	46	76.7%
Group 2 (Control ECE)	25	0	0	1	4	0	0	0	0	24	96%

N.B: All hatched chicks did not receive any medicine during 21 days observation period and were vaccinated against IBD, NDV.

Necropsy findings:

- **Dead embryos:** Congestion of the liver, myocardium and yolk sac.
- **Dead chicks (till 7 days old):** Enteritis, omphalitis, unabsorped yolk sac, distended gall bladder, congestion of liver, heart with septicaemic picture

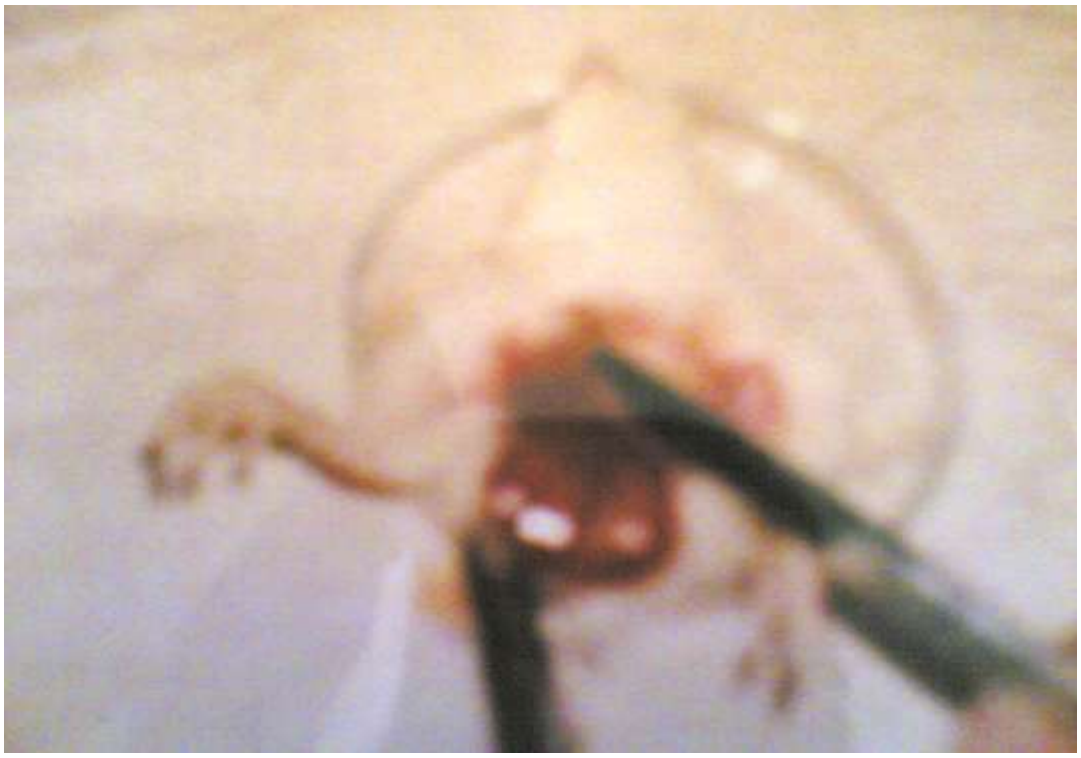

Fig.3: Two day-old *GR Aeromonas hydrophila* infected chick. Notice the congestion of liver, myocardium, yolk sac and septicaemic picture.

Table (2): Body weight gain of chicks weekly

Week / Chicks	Body weight gain (One chick / gram)		
	1ST Week	2nd Week	3rd Week
Group1 (Infected chicks)	93 gm	190 gm	320 gm
Group 2 (Control chicks)	98 gm	220 gm	423 gm
Difference	5 gm	30 gm	112 gm

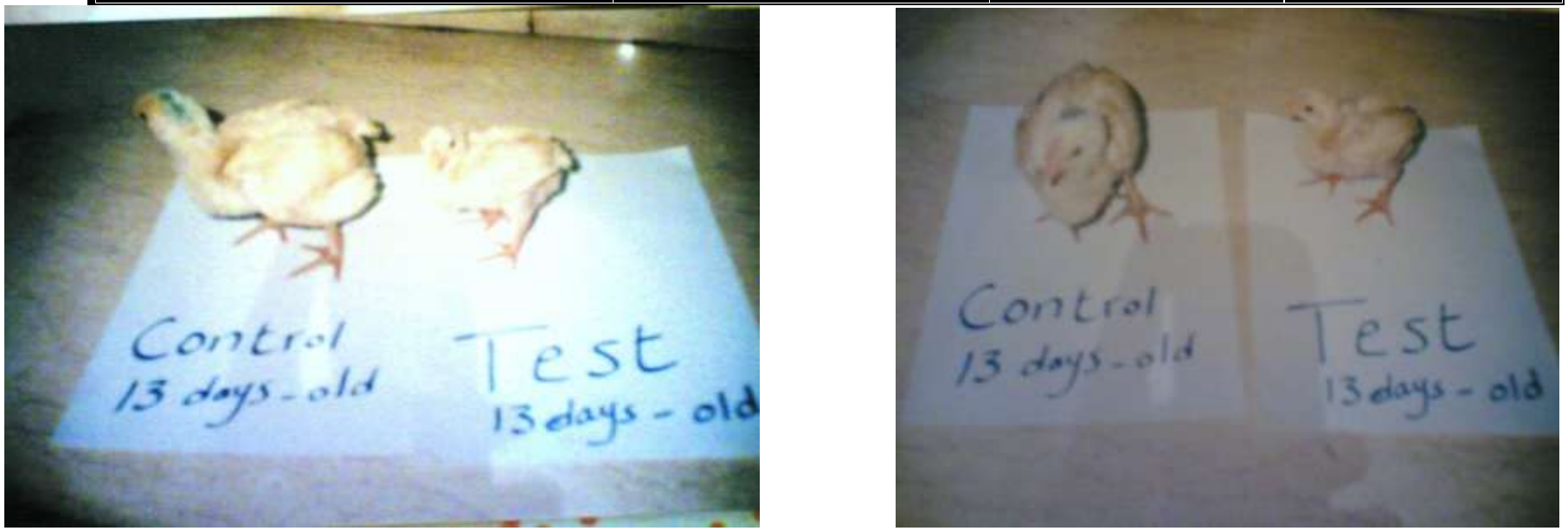

Fig. 4: Thirteen day-old experimentally infected chicks with *GR Aeromonas hydrophila* and control chicks. Notice the difference in body weight.

Table 3: Results of bacterial re-isolation of *GRAeromonas hydrophila* from infected 18–days-old dead ECE and sacrificed survived chicks.

Re-isolation from dead ECE		Re-isolation from survived chickens						
No. of dead ECE/Total No.	No. of Positive cases	No. of positive cases	Intestine	Liver	Heart	Spleen	Kidney	Lung
5/60	5/5*	46/46	44/46	12/46	4/46	2/46	1/46	2/46
8.3 %	100 %	100 %	96 %	26 %	9 %	4 %	2 %	4 %

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

Results of histopathological examination:

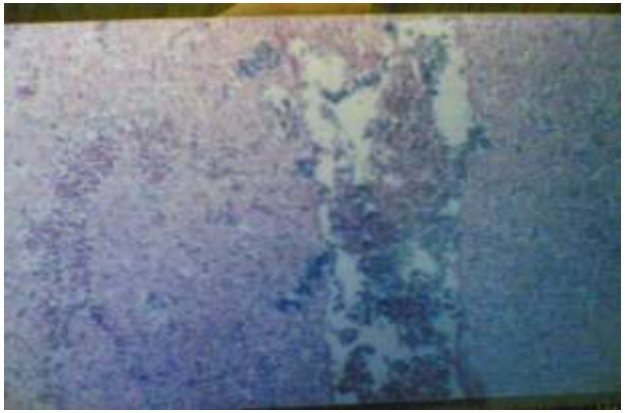

Fig. 5: Liver of dead-in shell embryo. Notice the severe dilatation of hepatic blood vessels in addition to mild hemorrhages.

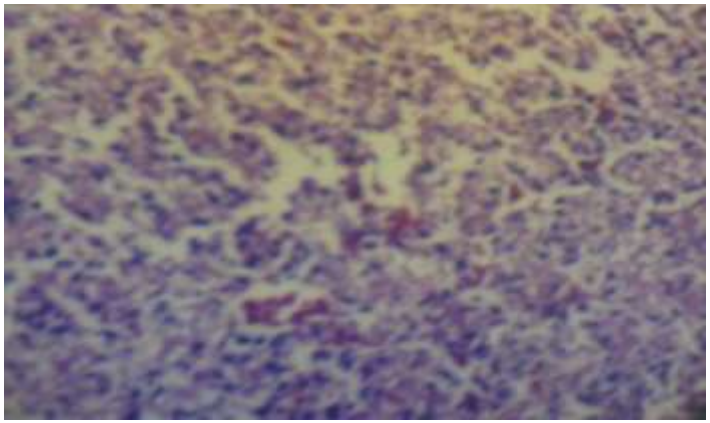

Fig. 6: Liver of infected dead one day-old chicks. Notice the mild congested blood vessels. All hepatic cells suffered form muscular degeneration.

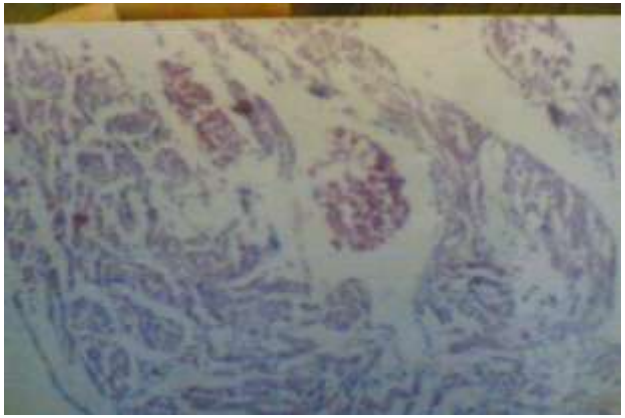

Fig. 7: Heart of dead one dat-old chicks. Notice the congestion of Coronary blood vessels with intramuscular oedema.

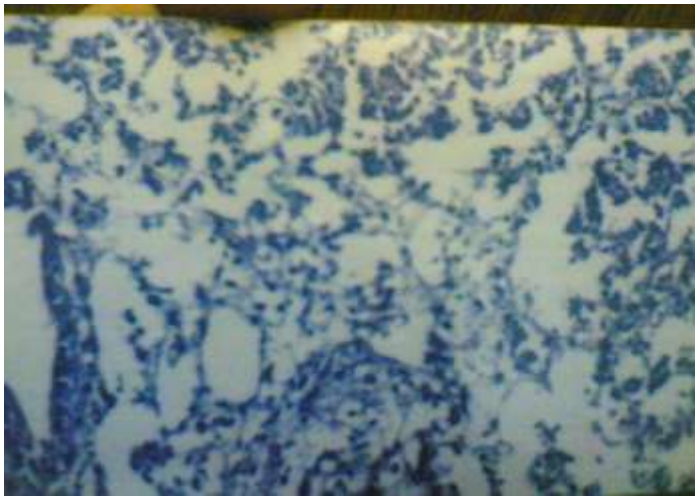

Fig. 8: Liver of dead chicks at 2 days old. Notice the dispersion of hepatocytes, some of hepatic lobules showed necrobiotic changes.

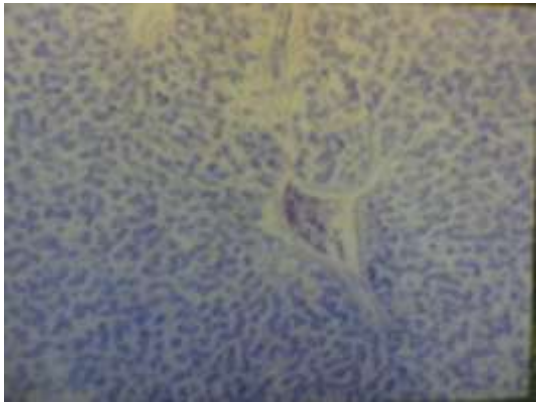

Fig. 9: Liver of sacrificed birds at 21 days old. Notice the pronounced oedema.

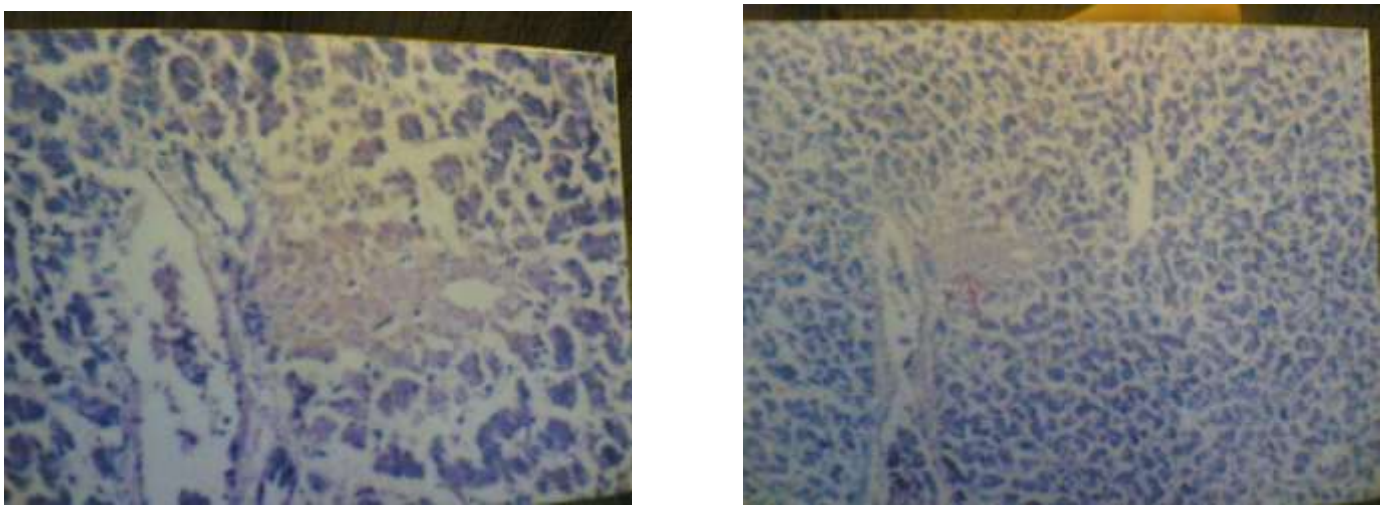

Fig.10: Liver of sacrificed infected birds at 21 days of age. Notice the pronounced oedema in addition to peripheral coagulative necrosis of the liver Cells

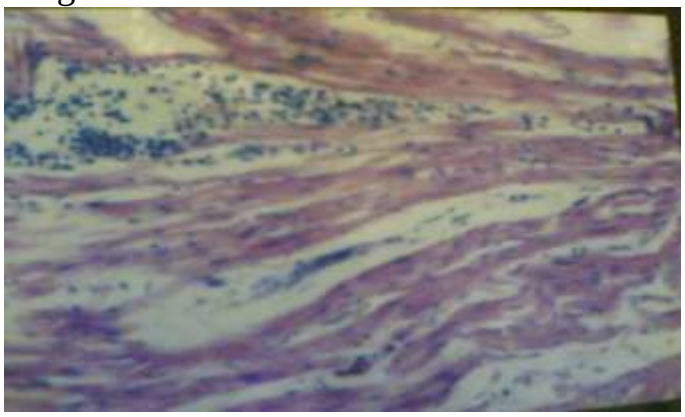

Fig. 11: Heart of 21 day-old infected sacrificed chickens. Notice that the cardiac muscle fibers were dispersed due to oedema with intramuscular aggregation of inflammatory cells (mainly lymphocytes and hepatocytes).

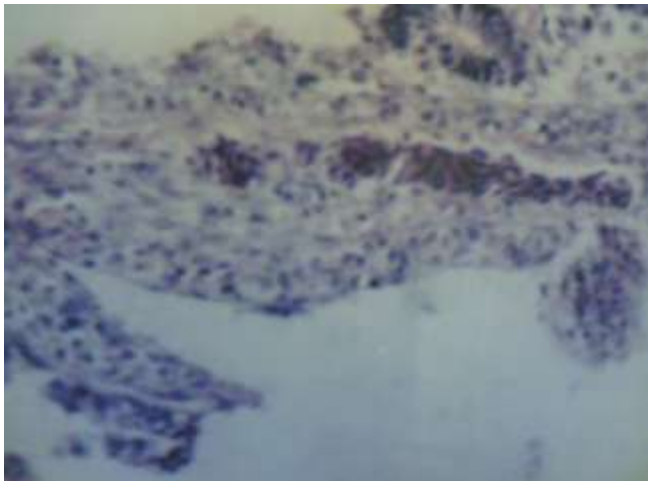

Fig. 12: Intestine of 21 day-old infected sacrificed chickens. Notice the hemorrhages in the villi.

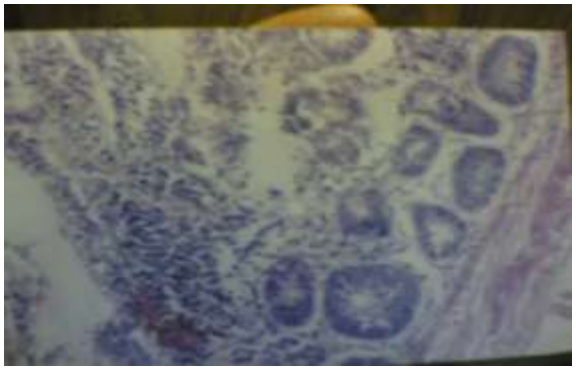

Fig. 13: Intestine of 21 day-old infected sacrificed chickens. Notice the infiltration of inflammatory cells in lamina propria.

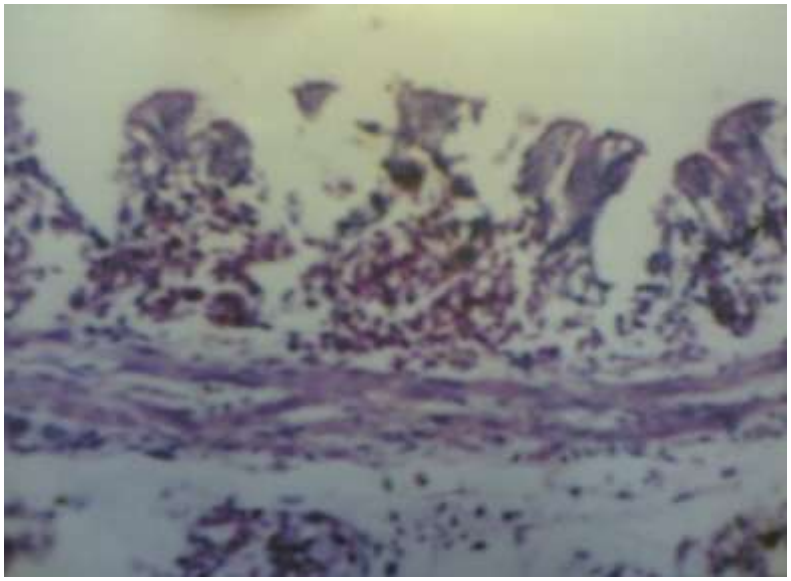

Fig. 14: Lung of 21 day-old infected sacrificed chickens. Notice the pulmonary oedeme, the pronounced alveolar congestion, and the tertori bronchioles showed subepithelial hemorrhage.

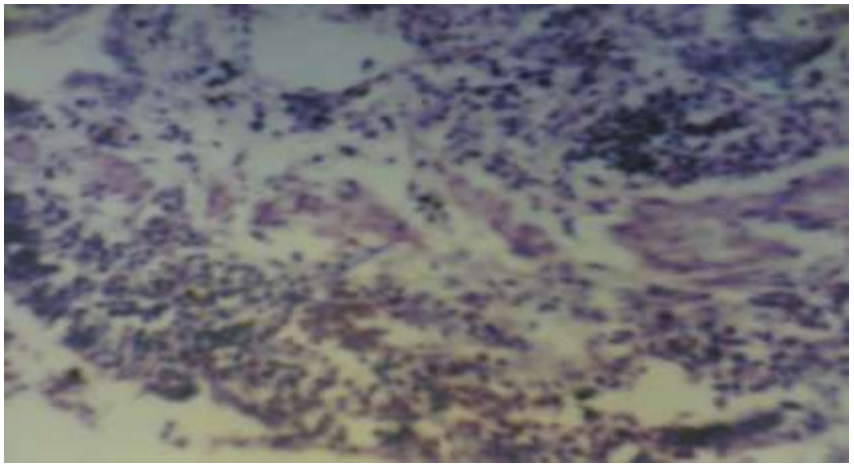

Fig. 15: Lung of 21 day-old infected sacrificed chickens. Notice the aggregation inflammatory cells mainly lymphocytes and heterophilis in the wall of bronchioles.

Experiment 2

The viability of *GR Aeromonas hydrophila* in drinking water under controlled laboratory conditions :

To each one litre of tap water pH6.7 kept in plastic water trough 5×10^9 viable bacterial cells of *GR A. hydrophila* broth culture were added per each ml. of water then kept at room temperature (25-40 C). Sampling was undertaken as follows; the first 12 hours every 3 hours, the second 72 hours-four times a day (every 6 hours), from 4^{th} to 7^{th} days twice a day, 8–14 days- daily, 15–21 twice a week and 3–7 weeks post inoculation–weekly. Culturing was done by Inoculation with one milliliter of tap water in a test tube containing 9 milliliters nutrient broth media containing 100 mg gentamycin/liter and was incubated at 30 ± 1^oC for 24 hours; then a loopful from resultant growth was streaked onto *Aeromonas* agar media and other streaked onto MacConkey agar plate. The plates incubated at 30 ± 1^oC for 24 hours.

Results:

GR Aeromonas hydrophila survived in tap water for 26 days and died thereafter.

Experiment 3

The viability of *GR Aeromonas hydrophila* in different materials under controlled laboratory conditions:

Sterile chicken crates, ration, faeces, saw dust and straw were used in this experiment. Two hundreds grams of each were inoculated with 5×10^9 viable bacterial cells of *GR A. hydrophila* broth culture per each gram then kept at room temperature (25-40 C). Samples were taken frequently in the following manner; day 1-3 twice daily, day 4-7 daily, day 8-23 every other day and day 24-45 weekly. Collected samples were inoculated into nutrient broth containing 100 mg of gentamycin / liter, incubated at 30 ± 1^{o}C for 24 hours then a loopful was streaked onto ***Aeromonas*** agar **media** and other streaked onto MacConkey agar plate. The plates were incubated at 30 ± 1^{o}C for 24 hours.

Result:

Aeromonas hydrophila remain viable for 11days, 9 days, 23 days, 22 days and 17 days in chicken crates, ration, faeces, saw dust and straw respectively.

Experiment 4

Study on faecal shedding, possible vertical transmission and effect of probiotic administration on *GR Aeromonas hydrophila* infection in chickens:

Thirty-four; 33 week-old chicken breeders consisting of 30 hens and 4 cocks were used in this experiment. The birds 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. Chickens of groups 1and 2 were orally infected with 2 ml of *GR A. hydrophila* (1.5×10^9 CFU/ml) on 2 successive days. Chickens of group 1 were fed on a ration containing 0.5 kg. / ton the probiotic" ***Pediococcus acidilactici"*** (Bactocell® produced by Lallimand Co., France) while those of group 2 were fed on a plain ration. Chickens of group 3 were subcutaneously infected with one ml of *GR A. hydrophila* containing 1.6×10^9 CFU. While birds of group 4 were kept without infection as blank control group.

Three days post infection (PI) cloacal swabs were collected daily during 1st week PI and every other day during 2nd week PI to study faecal shedding of *GR A. hydrophila*. Fertile eggs were collected from all groups to study egg shedding in the following manner:

One hundred and ten fertile eggs were collected from each group. These eggs were divided into 3 batches (1-3). Those of batches 1and 2 (25 eggs each) were subjected to bacteriological examination for reisolation of *GR Aeromonas hydrophila* from egg shell as well as from egg albumin and egg yolk respectively after **Shane and Gifford (1984)** as follows: The eggs were stored for 5 days at 4 degrees before the outer and the internal egg were cultured for *GR Aeromonas hydrophila*. For outer shell examination eggs were placed for 5 minutes in nutrient broth in plastic bags and the broth was incubated at 30 ± 1 ^{o}C for 24 hours before streaking on gentamycin Aeromonas agar and gentamycin MacConkey agar media. For internal examination yolk was cultured by swabbing the pointed end of the egg with 70% alcohol and then puncturing the shell with a sterile forceps while albumin was allowed to drain out without breaking the vitelline membrane. The vitelline membrane was cut with sterile scissors and 1 ml of yolk was collected with a syringe and incubated at 30 ± 1 ^{o}C for 24 hours in 15 ml of nutrient broth before streaking on gentamycin Aeromonas agar and gentamycin MacConkey agar media.

Eggs of batch 3 batch (60 eggs) was incubated for hatching and hatched chicks were kept under observation for 21 days for clinical signs and mortalities.

At the end of the experiment, All parent chickens were sacrificed and specimens were collected from ovary, intestine, heart, liver, spleen, kidneys, lungs, brain and different parts of oviduct (infundiblum, isthmus, uterus and vagina). The collected samples were subjected to bacteriological examination for determination of the localization sites of *GR Aeromonas hydrophila*. Sampling of organs for ***GR Aeromonas hydrophila*** was done as follows: Yolk collected on swab 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 taking care to avoid contamination from peritoneum. Similarly the exterior of the liver was seared and its interior was sampled in a sterilized swab that was then cultured, and as in spleen, heart, kidney, lung and brain moreover contents of the caecum was also cultured on nutrient broth and incubated at 30 ± 1 ^{o}C for 24 hours then streaked on on gentamycin Aeromonas agar and gentamycin MacConkey agar media.

Result:

Obtained results are shown in tables 4-5.

All samples taken from albumin, egg yolk and internal organs of parent chickens revealed negative result for re-isolation of *GR Aeromonas hydrophila.*

Table (4): Cloacal shedding of *GR Aeromonas hydrophila.*

OUP 1			GROUP 2			GROUP 3	
. OF MPLES	POSITIVE	PERCENT	NO. OF SAMPLES	POSITIVE	PERCENT	NO. OF SAMPLES	POSITIVE
	3	37.5%	8	8	100%	8	2
	1	12.5%	8	7	87.5%	8	1
	2	25%	8	8	100%	8	zero
	Zero	zero%	8	3	37.5%	8	zero
	1	12.5%	8	6	75%	8	zero
	Zero	zero%	8	2	25%	8	zero
	Zero	zero%	8	1	12.5%	8	zero

	Zero	zero%	8	2	25%	8	zero
	Zero	zero%	8	1	12.5%	8	zero
	Zero	zero%	8	Zero	zero%	8	zero
	Zero	zero%	8	Zero	zero%	8	zero

Table 5: Egg shedding of *GR Aeromonas hydrophila* (outer shell shedding).

OF LES	GROUP 1		GROUP 2		GROUP 3		CONTROL	
	POSITIVE	PERCENT	POSITIVE	PERCENT	POSITIVE	PERCENT	POSITIVE	PE
	1	4%	3	12%	zero	zero%	zero	zer

Other microorganisms were isolated lactose fermented and late lactose fermented from groups.

(6) isolates from G1, (14) isolates from G2, (3) from G3, and (9) from control G.

What is their significance ?

Discussion

A.hydrophila received an increasing attention as a food-born enteropathogen in human being which isolated from poultry eggs **(Varnam and Evans, 1991).** In domestic poultry, Aeromonas hydrophila was isolated from septicaemic condition of 3-16 weeks-old commercial turkeys, poultry faeces, haemorrhagic septicaemia in ducks, from faeces and carcasses of broiler chickens as well as from out breaks in duck folks suffered from sudden death with clinical signs of anorexia and dyspnoea **(Gerlach and Bitzer 1971; Stern et al., 1987; Jindal et al., 1993; FanDe et al., 1997; Akan et al., 1998; and KeMin et al., 1998).** This research was designed to clarify some epidemiological aspects on Aeromonas hydrophila infection in chickens. This was including; pathogenicity, cloacal shedding, survival of the organism under controlled laboratory conditions and possibility of vertical transmission. As *A. hydrophila* is sensitive to gentamicin **(FanDe et al., 1997; San et al., 1997; Kelley et al., 1998)**, for the epidemiological study; a gentamicin resistant *A. hydrophila* strain (*GR A. hydrophila* strain) was prepared for labeling purpose.

In experiment 1, the possible transmission of *GR Aeromonas hydrophila* via egg shell penetration (the in-ovo infection) was investigated.

The rate of hatchability reached 91.7 percent in infected group as compared to 100 percent in control one. A result that shows the responsibility of *Aeromonas hydrophila* for 8.3% embryonic mortality.

This could declare the role of the increased humidity and temperature as well as the poor hygienic hatchery conditions in provoking *A. hydrophila* infection via egg shell penetration. Dead embryos exhibited severe congestion of the liver, myocardium and yolk sac; while the survivors acquired omphalitis, septicaemia and enteritis. The chick mortality reached 13.3 % and 1.7 % during 1st week and 2nd week observation period as compared zero %, 4 % in non-infected controls respectively. Dead embryos suffered from septicaemia and congestion in all the internal organs with a rate of re-isolation of 100%. While the
survived infected chicks exhibited omphalitis, ruffling feathers, general weakness, inappetance, and enteritis. These results are in complete accordance with that reported by **Ocholi and Kalejaiye (1990)** who isolated *A. hydrophila* from liver, lung and intestine of ground hornbill suffering from haemorrhagic septicemia with haemorrhage in internal organs**. Gerlach and Bitzer (1971)** described a septicemic condition in commercial turkeys aged 3-16 weeks that was attributed to *A. hydrophila* infection with 10-30% morbidity and 1-5% mortality. **Saif and Busch (1974)** studied the synergistic relationship of salmonella infection and *A. hydrophila* in newly hatched poults and found that both organisms together produce 30% mortality but neither organisms produced mortality when inoculated individually.

The hatched chicks showed loss in their weights reaching 5, 30 and 112 grams at 1st, 2nd and 3rd weeks of age respectively when compared with their non-

infected penmates (Table 2). **Yadov and Verma (1998) and Kuthat et al. (2001)** reported on stunting of growth in chicks infected with *A. hydrophila*.

The rate of *GR A. hydrophila* re-isolation from dead embryos (yolk sac, liver, heart and intestine) reached 100 percent. While, in sacrificed survivors were 96 %, 26 %, 9%, 2 %, 4%, 2% and 4% intestine, liver, heart, spleen, kidney and lung was respectively. These findings accord with those described by different autors **(Aguirre *et al.;* 1992, Glunder and Siegmann; 1989, Shan *et al.*; 1984 and El-Gohary and Amal; 2002).**

Regarding results ***Aeromonas hydrophila*** infection resulted in the chicks Dead chicks during 1st week suffered the following clinical signs; dullness, inappitance and diarrhea, as well as showed moderate congestion in kidneys and intestine with slight congestion in liver, lung and yolk sac with severe enteritis and slight urates in ureters.

Further more the re-isolation of ***Aeromonas hydrophila*** from liver, heart, kidneys and lung can be explained by during septicemia.

In experiment 2, the survival of *A. hydrophila* in tap water was investigated under controlled laboratory conditions. The organism survived for 26 days at room temperature. This could reflect the long survival time of *A. hydrophila* in water which might result in the spread of infection within the flock. No available literature concerning the survival of *A. hydrophila* in drinking water. **Awaad (1975)** estimated the survival of *E.coli* in drinking water with metal and pottery containers as 36 and 99 hrs respectively. **Sarakbi (1983)** detected *Klebsiella pneumoniae* and *Klebsiella ozaenae* in drinking water for 9.5 and 6.5 days respectively and he mentioned that the material of the container did not provide a longer survival for the organism. **Rippey and Cabelli (1980)** studied the densities of *A. hydrophila* in various natural water of lrland. They reported that the bacteria seemed to be seasonally distributed with maximum count during summer through early fall. **Arcos et al., (1988)** recorded that *A. hydrophila* could be recover from polluted water. **Legnani et al., (1998)** studied the occurrence of *Aeromonas species* in drinking water supplies in a mountain area in northeast Italy and most of the isolates were identified as *A. hydrophila* and it was believed that the search for these micro-organisms should be adopted as a further indicator of drinking water quality. **Varnam (1991)** isolated *A. hydrophila* from chlorinated and unchlorinated piped water supplies. The organism has been isolated from bored well water.

In experiment 3, the viability of *GR A. hydrophila* was investigated in different material, simulating the flock condition to predict the mechanism of spread. The findings showed that *A. hydrophila* persisted in chicken crates, faeces, ration, saw dust and straw for 11days, 9 days, 23 days, 22 days and 17 days respectively. It could be concluded that the organism remains viable for a long period with possible allowing lateral spread. Reviewing the available literature it seems that viability of *A. hydrophila* in different materials is for the first time.

Awaad (1975) studied the survival of *E.coli* (chicken isolates) and found that they remained viable in meat for one week when kept at room temperature for 10 days at 4 ^{o}C and 25 days if kept at freezing temperature; the organism survived in chicken faeces for 30 days when kept at room temperature. **Sarakbi (1983)** investigated the survival of *Klebsiella organisms* in different material under control laboratory conditions and he pointed out that *K. pneumoniae* and *K. ozaenae* survived in chicken meat for 7, 20, 35 and 20 days when kept at 22 ^{o}C , 4 ^{o}C, - 4 ^{o}C and -20 ^{o}C respectively. *K. pneumoniae* survived in cloth, nylon, litter, faeces, saw dust and straw for 36, 105,42, 90, 15, 81, 120, 66, 60 and 90 days respectively; while *K. ozaenae* remained viable for 54, 120, 21, 102, 27, 105, 141, 72, 72, and 123 days in sequence listed before. **Glunder (1984)** mentioned that *Bortodella avium* survived best in dust and glass regardless the temperature and the author emphasized the role of air born infection. **Youseif (1989)** mentioned that *Proteus mirabilis* remained viable for 31days, 7 days, 31 days and 46 days in chicken crates, faeces, ration, saw dust and straw respectively.

Experiment 4 was designed to investigate the pattern of cloacal shedding of *GR A. hydrophila* in infected parent chickens during a period of 2 weeks (Table4). The cycle of infection in parent chickens infected with *GR A. hydrophila strain* was studied in this experiment. Faecal shedding of the inoculated organism revealed higher percentage in orally infected chickens than subcutaneously infected ones. However addition of probiotic to the ration of orally infected group (group1) resulted in lowering of this shedding. (Table 4). On studying shedding in fertile eggs collected from these chickens; egg shell contamination revealed 12 % in orally infected chickens (group 2). In orally infected and probiotic treated birds reached 4 % (group1). While no shedding of *GR A. hydrophila* was determined in subcutaneously infected birds. These results draw attention to the role of oral infection 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 (Table 5). All samples taken from albumin, egg yolk and internal organs of parent chickens on their sacrificing revealed negative result for re-isolation of *GR Aeromonas hydrophila*. It could be concluded that *A. hydrophila* is not congenitally transferred because it is not recovered from albumin and yolk. No clinical signs could be noticed in *GR Aeromonas hydrophila* infected parent chickensvia oral or subcutaneous routes, and the organism could not recovered from their internal organs at the end of the experiment. It could be concluded that *A. hydrophila* assumes a persistent nature during which a shedding occurs and the egg contamination takes place during the intestinal passage, therefore ovary and oviduct do not participate a role in dissemination of the infection.

Conclusions

This research project brought out the following conclusion:

1. ***Aeromonas hydrophila*** induces lowered hatchability.

2. ***Aeromonas hydrophila*** is considered to be a potential embryo pathogen producing septicaemia, unabsorbed yolk sac, enteritis and relatively high mortality in chicks.

3. It has been proved that ***Aeromonas hydrophila*** occurs as one of the egg contaminants via shell penetration during intestinal passage.

4. screening commercial eggs against ***Aeromonas hydrophila*** seems urgent.

5. Omphalitis, ruffled feathers, under body weight and bad feed conversion rate are associated with ***Aeromonas hydrophila*** infection.

6. ***Aeromonas hydrophila*** starts usually with an infected eggs and cycle proceeds thereafter unless thorough control measures are adopted.

7. ***Aeromonas hydrophila*** survives for several weeks in the contaminated water, litter and ration; therefore the feed prophylaxis, clean water new litter as an alternative can be overlooked.

8. ***Aeromonas hydrophila*** may infect birds by oral route and had colonized the intestine as a part of intestinal flora.

9. ***Aeromonas hydrophila*** is not congenitally transferred.

Summary

the in-ovo infection with ***Aeromonas hydrophila*** was investigated. Sixty chicken embryo aging 18 days were dipped in a suspension containing 10^9 viable bacterial cells and twenty five eggs were left as a control. Dead embryos were necropsied and attempt for reisolation was done. The hatched chicks were kept till the 21 days old to clinical signs, mortality rate, and reisolation from several organs of dead chicks, histopathological examination, and weight gain. After 21 days old chicks were sacrificed to re-isolation from several organs and histopathological examination were done.The results of the 18 day old eggs were 91.7 percent hatchability and 100 percent hatchability in control group. Accordingly, it seems that ***Aeromonas hydrophila*** inducing an adverse effect on hatchability.The ***Aeromonas hydrophila*** infection produced relatively high embryos mortality found that dipping of embryos at the later stages with an active suspension of ***Aeromonas hydrophila*** produce death of the entire hatch within 72 hours. It seems that the increased humidity and temperature favoured the egg shell invasion by ***Aeromonas hydrophila;*** and the role of poor hatchery conditions in provoking ***Aeromonas hydrophila*** infection.Dead embryos exhibited severe congestion of the liver, myocardium and yolk sac; while the survivors acquired omphalitis, septicaemia and enteritis.The reisolation rate from dead embryos from yolk sac, liver, heart and intestine was 100 percent and from several organs of survivor's intestine, liver, heart, spleen, kidney and lung was 96 %, 26 %, 9%, 2 %, 4%, 2% and 4% respectively. The survivors exhibited omphalitis, ruffling feathers, general weakness, inappetance, and enteritis as well as lowered in body weight was in 1^{st} , 2^{nd} and 3^{rd} week 93 gm, 190 gm and 320 gm respectively while in control group was in 1^{st}, 2^{nd} and 3^{rd} week 98 gm, 220 gm and 423 gm respectively.

The results revealed that embryonic mortality reaching to 8.3% in ECE and chick mortalities at 1^{st} week and 2^{nd} week reaching to 13.3% and 1.7% respectively. compared with control group zero%, 4% and zero% respectively.

Dead embryos were suffering from septicaemia and congestion in all the internal organs with the rate of re-isolation in 100%.As regard to results ***Aeromonas hydrophila*** infection resulted in the chicks dead in the 1^{st} week suffering from dullness, inappitance and diarrhea. Postmortem lesions were moderate congestion in kidneys and intestine with slight congestion in liver, lung and yolk sac there was severe enteritis with slight urates in ureters. Further more the re-isolation of ***Aeromonas hydrophila*** from liver, heart, kidneys and lung can be explained by during septicemia.

The survival of ***Aeromonas hydrophila*** in the tap water was searched under controlled laboratory conditions. The organism survived for 26 days at room temperature. This could reflect the long survival of ***Aeromonas hydrophila*** in water which might result in the spread of infection within a flock.

In experiment three, the viability of ***Aeromonas hydrophila*** was investigated in different material, simulating the flock condition to predict the mechanism of spread. The findings showed that ***Aeromonas hydrophila*** persisted in chicken crates, faeces, ration, saw dust and straw for 11days, 9 days, 23 days, 22 days and 17 days respectively.We concluded that the organism remain viable over along period possibly allowing the lateral spread; death occurred soon faeces while it withstand in the saw dust and ration.

In experiment four:

Study of Cloacal shedding of G.R strain of ***Aeromonas hydrophila*** was studied for a period of two weeks.

- in group one the ration containing probiotic Bactocell the persistent shedding of organism was proved from 3 – 7 days PI.
- in group two the persistent shedding of organism was proved from 3 – 15 days PI.
- in group three the persistent shedding of organism was proved from 3 – 4 days PI.

higher re-isolation rate from Cloacal swabs was reflected that ***Aeromonas hydrophila*** may infect birds by oral route and had colonized the intestine as a part of intestinal flora i.e. the pattern of Cloacal shedding was decreased in group one when probiotic was added to ration.

The cycle of infection of chickens with GR strain of ***Aeromonas hydrophila*** was studied in experiment 4. Layer chickens and their eggs were kept under observation during which clinical parameters and bacteriological investigation were recorded.The egg shedding of GR strain of ***Aeromonas hydrophila*** outer shell contamination The findings showed that eggs received outer shell contamination from dams during the period of intestinal shedding. It could be concluded that ***Aeromonas hydrophila*** (GR strain) is not congenitally transferred because it is not recovered from albumin and yolk as well as intestine and other internal organs of layers at the end of the experiment specially ovary and different parts of oviducts.

It could be concluded that ***Aeromonas hydrophila*** assumes a persistent nature during which a shedding occurs and the egg contamination takes place during the intestinal passage, therefore ovary and oviduct do not participate a role in dissemination of the infection.

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بعض الجوانب الوبائية لعدوى ميكروب الايروموناس هيدروفيلا في الطيور

رسالة مقدمة من

طبيب بيطري : أحمد فتحي عبد اللطيف عيد

بكالوريوس العلوم الطبية البيطرية 1987

دبلوم أمراض الدواجن والأرانب 2002

للحصول علي درجة الماجستير في العلوم الطبية البيطرية

(أمراض الدواجن)

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الملخص العربي

التجربة الأولى:

تم استبيان إمكانية العدوى بعترة خاصة من صنف الايروموناس هيدروفيلا.

تمت عدوى عدد 60 أجنة بيض محضنة لعمر 18 يوم بتغطيسها بمعلق الميكروب المحتوي على 10^9 خلية بكتيرية نشطة وتركت عدد 25 أجنة بيض ضابط للتجربة وقد تمت ملاحظة البيض حتى الفقس. الأجنة النافقة حتى عمر الفقس تم تشريحها وخضعت لإعادة العزل البكتيري والفحص الهيستوباثولوجي.

وتمت ملاحظة الكتاكيت الفاقسة حتى عمر 21 يوم من خلال الأعراض الإكلينيكية ومعدل النفوق والوزن المكتسب والعزل البكتيري من الأعضاء الداخلية والفحص الهيستوباثولوجي للكتاكيت النافقة وفي نهاية الفترة (عمر 21 يوم) تم ذبح الكتاكيت وتم إعادة العزل البكتيري من الأعضاء الداخلية والفحص الهيستوباثولوجي ومقارنة الأوزان المكتسبة بين الضابط وكتاكيت التجربة وكانت النتائج كالتالي:

1 – زيادة النفوق الجنيني حتى عمر الفقس 8.3 % بينما لايوجد نفوق جنيني في البيض الضابط أي أن نسبة الفقس 91.7 % وعليه ميكروب الايروموناس هيدروفيلا يسبب نفوق الأجنة خلال 72 ساعة مما يخفض نسبياً نسب الفقس كما تم العزل البكتيري للميكروب من كيس المح والكبد و القلب والطحال والأمعاء كما وجد احتقان شديد في الكبد والقلب وكيس المح في الأجنة النافقة كما أن نتائج الفحص الهستوباثولوجي وجد تمدد شديد في الأوعية الدموية للكبد بالإضافة لوجود أنزفه طفيفة مع أن باقي الأعضاء وتشمل القلب والأمعاء والكلى ليس بها آفات تشريحية خاصة للمرض.

وفي كتاكيت التجربة حتى عمر 21 يوم وجد النافق بنسبة 23.3 % بينما في الكتاكيت الضابط 4 %.كما تم عزل الميكروب من الأعضاء الداخلية من الكتاكيت النافقة .

كما تم عزل الميكروب من الأعضاء الداخلية من الكتاكيت الحية من الأمعاء و الكبد والقلب والطحال والكلى والرئتين كانت بنسب 96 % و 26 % و 9 % و 2 % و 4 % و 2 % و 4% على التوالي مع أعراض إكلينيكية التهاب سره وانتفاش للريش وضعف عام وضعف للشهية مع اسهالات بجانب انخفاض في وزن الجسم المكتسب في الأسبوع الأول والثاني والثالث كانت الأوزان 93 جم و 190 جم و 320 جم على التوالي بينما في كتاكيت الضابط 98 جم و 220 جم و423 جم على التوالي.

وقد أسفرت النتائج على نفوق جنيني وصل إلى 8.3 % ونفوق في الأسبوع الأول والثاني وصلت إلى 13.3 % و 1.7 % على التوالي مقارنة بالضابط 0.0 % و4 % و 0.0 % على التوالي .

التجربة الثانية:

إمكانية بقاء ميكروب الايروموناس هيدروفيلا في ماء الصنبور.

تحت الظروف المعملية الميكروب يمكنه البقاء حياً في ماء الصنبور حتى 26 يوم في درجة حرارة الغرفة وهذا يعكس طوال فترة البقاء حياً للميكروب في الماء التي قد تؤدي إلى انتشار العدوى داخل القطيع.

التجربة الثالثة:

دراسة إمكانية بقاء ميكروب الايروموناس هيدروفيلا في بعض المواد التي تتواجد في مزارع الدواجن والتي قد تلعب دور في انتشار العدوى بين الطيور . فقد أظهرت النتائج أن ميكروب الايروموناس هيدروفيلا يمكنه البقاء حياً في كراتين الكتاكيت وزرق الطيور و العلف ونشارة الخشب والقش لمدة 11 يوم و 9 أيام و23 يوم و22 يوم و17 يوم على التوالي. ونلاحظ أن الميكروب يموت في فترات زمنية صغيرة في زرق الطيور ويظل لفترات طويلة في العلف ونشارة الخشب ونستنتج من ذلك أن الميكروب يظل حياً لفترات طويلة نسبياً مما يساعد على انتشار العدوى الأفقية .

التجربة الرابعة:

1) **تم استبيان الإفراز المجمعي لميكروب الايروموناس هيدروفيلا المقاوم للجنتاميسين وحتى الأسبوع الثاني بإجمالي عدد 264 مسحة مجمع بداية من اليوم الثالث بعد العدوى. ووجدت النتائج كالتالي.**
   - **في المجموعة الأولى من الدجاج والتي تمت العدوى بها عن طريق الفم والتي يتم تغذيتها على علف يحتوي على بربييوتيك ظل الإفراز المجمعي للميكروب من اليوم الثالث وحتى اليوم السابع بعد العدوى.**
   - **في المجموعة الثانية من الدجاج والتي تمت العدوى بها عن طريق الفم وتم تغذيتها على علف لايحتوي على بربييوتيك ظل الإفراز المجمعي للميكروب من اليوم الثالث وحتى اليوم الحادي عشر بعد العدوى.**
   - **في المجموعة الثالثة من الدجاج التي تمت العدوى بها عن طريق الحقن ووتم تغذيتها على علف لايحتوي على بربييوتيك ظل الإفراز المجمعي للميكروب من اليوم الثالث وحتى اليوم الرابع بعد العدوى.**

أعلى معدلات للعزل البكتيري للميكروب من الإفراز المجمعي في المجموعة الثانية والذي يرجح أن الدجاج قد يأخذ العدوى عن طريق الفم ويقوم بعمل مستعمرات في الأمعاء كجزء من الميكروبات التي تعيش في الأمعاء (flora) وذلك لأن الإفراز المجمعي قد قل بطريقة ملحوظة في المجموعة التي تم تغذيتها على علف يحتوي على بروبيوتيك.

2) **تم استبيان إفراز ميكروب الايروموناس هيدروفيلا المقاوم للجنتاميسين في الدجاج البياض من حيث التلوث الخارجي لقشرة البيض والإفراز الداخلي في البيض وانتقال العدوى الرأسية من الأم للكتكوت الناتج**

قد تمت الدراسة على متابعة الحالة الصحية للدجاج البياض والبيض الناتج والعزل البكتيري وتم تسجيلها

النتائج التي أسفرت عن الآتي:

- **أن البيض الناتج يتم تلوث القشرة الخارجية له في الأمعاء خلال فترة الافراز للميكروب.**
- **لاينتقل الميكروب رأسياً من الأم إلى البيض حيث أنه لم يتم عزله من بياض البيض أو المح بالإضافة إلى الأعضاء الداخلية للدجاج في نهاية الدراسة وخاصة من المبيض والأجزاء المختلفة لقناة البيض.**
- **ميكروب الايروموناس هيدروفيلا يتم افرازه في الامعاء وتتلوث القشرة الخارجية للبيض خلال مروره فترة افراز الميكروب وعلى ذلك فإن المبيض وقناة البيض لاتشترك في إفراز الميكروب ولاتلعب دوراً في نقل العدوى للبيض.**