Aeromonas Infection in Avians

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Abstract: The genus *Aeromonas* is a Gram-negative, rod-shaped, facultative anaerobic bacterium. Most members of the genus are mesophiles with an optimal growth temperature of 28°C. Some *Aeromonas* can grow at temperatures ranging from 4°C to 42° C, the capacity to grow at such extreme temperatures varies among strains and seems to be closely related to the source of isolation, or to environmental adaptation. *Aeromonas* isolates are belonged to 3 major groups; *A. caviae* group includes *A. caviae*, *A. eucrenophila* and *A. media*, *A. hydrophila* group includes *A. hydrophila* and amotile biogroup of *A. salmonicida* while *A. sobria* group includes *A. sobria* and *A.veronii*. The incidence of infection in Egypt reached to 25% with high morbidity. In other countries the infection reached to 86%. The induced pathology and virulence of organisms resulted from many factors including stress responses. *A. hydrophila* which adhere to epithelial cells are believed to colonize, produce lesions, therefore the interaction with the epithelial cells is the first step towards pathogenicity. The pathogenicity is associated with the liberation of virulence factors and cell associated endotoxin. The detection of the presence of such virulence factors is a better indicator of the potential risk for their pathogenicity. In12 day-old chicken embryos and adult Japanese quail *A. hydrophila* causes depression, ruffled feathers after 2 days post inoculations, severe diarrhea, emaciation, no specific lesions were observed after post mortem (PM) examination. Only congestion and friable livers were evident. In chickens it causes gastrointestinal disturbance. Post mortem lesions showed general congestion of all carcasses. In severely emaciated cases the lesions were confined to the intestine, which is filled with watery fluid and distended with gas. Infected chicken organs showed focal coaggulative necrosis in liver with mononuclear cells infiltration. Experimentally infected chicks with *A. hydrophila* were died acutely, while chicks died late demonstrated a transitory period of depression characterized by ruffled feathers and pasty vent before death. The organism was also isolated in pure culture from a pet parrot with bilateral conjunctivitis. *A. hydrophila* was reported to be the cause of the acute death of aviary canaries, and was also isolated in pure culture from a toucan with acute nephritis. In Ostrich the infection associated with severe necrotizing enteritis and septicemia in 10-yr-old male, while in commercial turkeys; Flock morbidity ranges between 10%-30% and mortality 1%-5%. Aeromoniasis is characterized by high morbidity and lower hatchability, under body weight with bad feed conversion. Susceptibility Patterns of *A. hydrophila* to antimicrobial agents varies to be more sensitive to gentamyicin, kanamyicin, chloramphenicol and tetracycline while resistance to ampicillin and penicillin. Diagnosis of Aeromoniasis in Avian is based on isolation and identification of pathogens**.** Detection of pathogenicity of the isolated strains *in vitro* and *in vivo* condition is an important step is dot ELISA and ELISA technique act as another efficient method in diagnosis of disease. The prevention and control of the disease is based on isolation and treatment of infected cases beside the hygienic measures in drinking water and addition of probiotic in ration.
[Zeinab M. S. Amin Girh. **Aeromonas Infection in Avians.** *Rep Opinion* 2018;10(2):36-40]. ISSN 1553-9873 (print); ISSN 2375-7205 (online). http://www.sciencepub.net/report. 5. doi:10.7537/marsroj100218.05.

Keywords: Aeromonas; Infection; Avians; ampicillin; penicillin

Introduction

Members of genus *Aeromonas* are autochthonous in aquatic environments. They are Gram negative, oxidase positive, ampicillin resistant with an exception; *A. trota* ***(Albert et al., 2000)***, non-spore forming, facultative anaerobic rods with a length between 1.0-3.5 µm and diameter between 0.3-1 µm. ***(Percival et al., 2004)***. The genus *Aeromonas* has undergone a number of taxonomic and nomenclature revisions over the past 20 years. Only five species of *Aeromonas* were recognized 15 years ago ***(Janda and Duffey, 1988)***, three of which are (*A. hydrophila, A. sobria,* and *A. caviae*). The mentioned species are existed as phenospecies containing multiple DNA groups, which could not be distinguished from one another by simple biochemical characteristics. Subsequent systematic investigations have resulted in the number of valid published genomospecies rising to 14 ***(Joseph and Carnahan, 2000).*** *Aeromonas* Infection is probably perpetuated by fecal shedding of *Aeromonas* species which can be persist in moist soil and water.

The contamination of chicken carcasses with motile *Aeromonas* species comes during the slaughtering process and the spread of motile Aeromonads from intestinal contents to carcasses via processing water ***(Akan et al., 1998; Sarimehmetoglu and Kuplulu, 2001). Lin et al (1996)*** *Aeromonas* is

among the environmental bacteria that can be recovered from dead -in –shell embryos and weak chicks. The disease in Avians was studied by many authors ***(El-Khashab,2001; Awaad et al., 2011)*** and concluded that most apparant symptoms and lesions are depression, ruffled feathers, sever diarrhea, emaciation, omphalitis, enteritis and septicaemia. No specific lesions were observed although general venous congestion as well as congestion of liver, spleen, lungs, kidneys, intestine. Duodenum showed sever hemorrhagic enteritis.

***Aeromonas* infection in chickens:**

Incidence of *Aeromonas* in Chickens:

Mohamed (2007) has isolated 45 isolates from 300 chickens (15%) of different ages in upper Egypt. On the other hand ***Mahmoud and Tanios (2008)*** have isolated 17 isolates of *A.hydrophila* from 250 commercial broiler chicks with a percentage of 6.8. 88.24 % of *A. hydrophila* isolates were positive for exotoxin assay and congo red binding test, while 52.94 % were positive for crystal violet binding activity**.**

Glunder (1988) isolated *A.hydrophila* from 80 birds from total 2236 ones. He found that monoinfection was found in 4 cases, while in all other cases *A.hydrophila* infection was combined with the presence of Enterobacteriaceae and or *Streptococci* and *Staphylococci*. ***Akan and Diker (1996)*** isolated *Aeromonas* species from chicken faeces. They added that *A.hydrophila* was the most prevalent in these samples. ***Yucel and Ctak (2003)*** detected the motile *Aeromonas* species; *A. hydrophila, A. caviae and A. sobria* with a total percentage of 86.9 from chicken samples. The dominant species were *A. hydrophila* and *A. sobria*. *A. hydrophila* was the predominant species in both raw and cooked meat products, followed by *A. sobria*.

Clinical signs of *A. hydrophila* infection in chicks:

Shane and Gifford (1985) reported that experimentally infected chicks were either died acutely without showing premonitory signs or after a transitory period of depression characterized by ruffled feathers. ***Efuntoye (1995)*** found that depression, ruffled feathers after 2 day post-inoculation, while sever diarrhea, emaciation for 12th day- old chicken and adult Japanese quail. No specific lesions were observed on post mortem examination although congestion and friable livers were evident. ***Awaad, et al. (2011)*** mentioned that survived infected chicks exhibited signs and lesions of omphalitis, enteritis and septicaemia with decrease in its weight gain.

Post Mortem Lesions:

Shane and Gifford (1985) revealed that no specific lesions were observed, except general venous congestion was evident. The Lesions were focal cerebral plaques and petechical hemorrhage on the mucosa of proventriculus and jejunum. Also pulmonary congestion and hepatic petechiae were observed. ***EL-Khashab (2001)*** observed a generalized s/c venous congestion as well as congestion of liver, spleen, lungs, kidneys, intestine especially duodenum showed sever hemorrhagic enteritis and liver showed streaks of hemorrhage in experimentally infected chicks.

Histopathology:

Shane and Gifford (1985) recorded comprised sever multi focal a cute coagulation necrosis of the neuropil with congestion of liver and extensive pulmonary necrosis. ***Mahmoud and Tanios (2008)*** detected the bacilli of organisms inside the hepatocytes and macrophages with marked cellular changes in yolk sac and s.c infected 1-day old chicks. In an attempt, there were marked degenerative and necrobiotic changes were observed in both hepatic and splenic tissue, beside muscular lesions manifested by hemorrhage, degeneration, oedema and myositis. In sever infected cases, the lesions were more severe and characterized by diffuse areas of necrosis in hepatic tissue, thrombus formation in the blood vessels together with large number of bacterial colonies and bacilli in the hepatic tissue. Marked muscular necrosis and myophagia were also noticed.

***Aeromonas* infection in other Avian:**

In Water fowl:

Korbel and Kösters (1989) found high mortality among water fowl at several locations From July to September 1988 and in all cases, an *Aeromonas hydrophila* infection was diagnosed. ***Feare et al. (1999)*** detected higher prevalences of *A. hydrophila* underscoring the observation in geese that may largely reflect local environmental contamination as well as acting as disseminators of pathogenic agents. The infected ducks with *A. hydrophila* showed salpingitis, septicemia, and airsacculitis. ***Zbikowski et al. (2006)*** reported epidemic deaths of mallard ducks after infection with *A.hydrophila*.

In wild and pet birds:

A. hydrophila was detected in acute death of aviary canaries (*Serinus canarius*), and also isolated in pure culture from a toucan (*Ramphastos toco*) with acute nephritis, and from a cockatiel (*Nymphicus hollandicus*) with chlamydiosis (psittacosis) (***Panigrahy et al., 1981).*** *Aeromonas hydrophila* was isolated from (faecal swabs and internal organs at post mortem examination) from wood ducks (*Aix sponsa*), Coscoroba swan (*Coscoroba coscoroba*), fulvous whistling ducks (*Dendrocygna bicolor*), puna teal (*Anas puna*), ringed teal (*Callonetta leucophrys*), black-necked swan (*Cygnus atratus*) and domestic goose (*Anser anser domesticus*) in Louisiana, USA ***(Shane and Gifford, 1985)***.

Glünder (2002) reported that the determination of *A. hydrophila* in nearly 3500 wild and pet birds providing statistically significant evidence in the composition of the intestinal flora that may depend on dietary habits.

In Ostrich:

França et al (2009) has reported that *Aeromonas* species were associated with severe necrotizing enteritis and septicemia in 10-yr-old male ostrich was diagnosed. The bird was in appetent for 3 wk and had neurologic signs 2 days prior to death. Macroscopically, no significant lesions were noted aside from congestion of the liver, kidneys, and spleen.

In turkeys:

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

Antibiotic susceptibility:

The susceptibility Patterns of *A.hydrophila* to antimicrobial agents have varied, but isolates were usually susceptible to chloramphenical, tetracycline and trimethoprin-sulfamethoxzol and relatively resistant to penicillin, polymixin and cephalasproins, ***Fass and Barnishan (1981) and Davis et al. (1978).*** In vitro susceptibility of *A.hydrophila* isolates to a variety of antibiotics, ***Soliman (1988)*** revealed that most of the *A.hydrophila* isolates are sensitive to chloramphenicol, nalidixic acid, streptomycin, kanamycin and colistin, while all of them were resistant to ampicillin and novobiocin.

Adeleke and Omafuvbe (2011) isolated *Aeromonas* from the poultry faeces obtained from the Obafemi Awolowo University Teaching and Research Farms, Ile-Ife, Nigeria. The antibiotic sensitivity patterns of the isolated bacteria against amoxicillin, augmentin, ceftriaxone, chloramphenicol, ciprofloxacin, erythromycin, gentamycin, nitrofurantoin, ofloxacin, pefloxacin, streptomycin, tetracycline, cotrimoxazole were determined. The quinolones (ofloxacin, ciprofloxacin and pefloxacin) were the most effective of all the antibiotics used. To the rest, The *Aeromonas sp.* showed a low-level antibiotics resistance.

Diagnosis:

The traditional culture techniques for direct isolation and identification of pathogens are available but they are usually time taking and laborious ***(Rathore et al., 2005)***. Also detection of the pathogenicity of isolated strains in *vitro* and *in vivo* conditions is an important. Identification using both phenotypic and genotypic methods based on biochemical studies, enzymatic assay and phylogenetic analysis of 16S rRNA sequences. The polyclonal antibody raised against the outer membrane protein of *A. hydrophila* was used in the detection of pathogen through dot ELISA technique. Recently, sequencing of 16S ribosomal RNA gene was proved to be stable and specific marker for bacterial identification ***(Martinez-Murcia et al., 2008; Chan et al., 2011)***. In addition, dot ELISA technique act as another efficient (specific and sensitive), simple to use and rapid diagnosis technique for the detection of aetiological agent of diseases ***(Swain et al., 2001).***

Prevention and control:

A. hydrophila infection in ECE is a potent pathogen that must be considered in planning strategies for control measures and biosecurity in hatcheries. Moreover, it is considered as a public health hazard ***(Youseif and Hassan 2003)***.

Antibiotic can be use but should be taken in consideration that resistance among *A. hydrophila* isolates from broiler chickens was higher ***(Shinde, et al. 2005 and Mohamed and Mohamed, 2012).***

Addition of probiotic must be put in consideration when control and prevention of Aeromoniasis is adopted. ***Awaad, et al. (2011)*** found that 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.

Prevention or sanitation of fish meal is studied by ***Mohamed and Mohamed (2012)*** and they have found two common clones between strains of fish meal and broiler chickens isolates, that provides a suggestive evidence of successful colonization and infection by particular strains of *A. hydrophila* after transmission from fish meal to broiler chickens. Hence, continuous monitoring of fish meal is an important to identify potential pathogenic *A. hydrophila* before its addition to food ration to reduce the risk of infection.

Vaccination is an important strategy in the control of this disease caused by *A. hydropila* among farmed fish ***(Asha et al., 2004).***

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2/18/2018