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Aeromonas Hydrophilla in Cultured Oreochromis Niloticus and its Effect on Economic Returne of Fish Farm Production

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***Abstract*—** The authors isolated Aeromonas hydrophilla (Biovar I, II, III, IV, and V) from a broad stock of Oreochromis niloticus with 65 % mortality in the winter of 2008, 2009 following their wintering season . There were hemorrhagic patches on the skin, fins, in the mouth and internal organs of affected fish. The bacterial characteristics of the isolated bacteria were studied; experimental infection succeeded only when the bacteria was applied by injection but not via water. Following experimental intraperitoneal infection with the isolate Aeromonas hydrophilla strains No. 5. haemorrhagic patches appeared allover the body of the planktonophagous fishes .
The microorganisms were highly virulent to all tested Oreochromis niloticus, as measured in terms of LD_{50} values. The results of Histopathology revealed the presence of dermal and subdermal hemorrhages, congestion of haemopoeitic organs and the presence of high numbers of immature erythrocytes in the blood vessels. The important role of stress factors in the pathogenesis of the disease was discussed. The economic analysis revealed that the Aeromonas hydrophila causes a great economic losses to fish production farms as its causes a high mortality among the infected fish that reached to 65 % , with decreasing the weight of the fish sale that may reached to 162.5 Kg/100 fish with decreasing the economic returns by 1625 LE/100 fish.

Keywords-; Aeromonas hydrophilla , Oreochromis niloticus , haemorrhagic patches , haemopoeitic organs

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I. INTRODUCTION

Fish diseases due to bacterial pathogenic are the major problems in aquaculture as they are found naturally in fish environment and under certain stress conditions cause severe economic losses to fish farms ***(Post, 1987 and Olsson et al., 1998).***

O. niloticus is the most common fish cultured in Egypt is farmed in polyculture with the Common carp (Cyprinus carpio L.), and mugilids in earthern pond (Abdelghany and Ahmed, 2002) .

Except the work of Bejerano et al. (1979), on Aeromonas hydrophilla infection, Faisal and Easa (1987), on Aeromonas hydrophilla and Plumb (1994) who mentioned the Aeromonas hydrophilla produce clinical signs similar to those observed in Pseudomonas and Flexibacter columnaris in tilapia and other infected fish species. In this study, an epizootic among Oreochromis niloticus caused by a Aeromonas hydrophilla is described.

Aeromonas hydrophila and other Aeromnads are among the most common bacteria in freshwater habitats throughout the world. They are necessarily present in normal microflora and hydrobionats inhabiting fish reservoirs (Kompanets et al., 1992). There are some potential risk factors associated with the main diseases of fish such as season and water temperature (Ortega et al., 1995). Mortality among high thermal stressed fish was 80% due to Aeromonas. (Noga, 1996).

However, they frequently cause problems in both feral and cultured fish (Cipriano, 2001) it is responsible for heavy economic losses caused by both high mortality and deterioration of product quality (Groff and Lapatra, 2000 and Karunasagar and Otta , 2003).

Aeromonas hydrophila, considered as one of the most important bacterial pathogens that causes a great economic losses to fish of either fresh or marine fish due to a high mortality with decreasing the fish weight and sale with decreasing of fish farm returns. (Alicia et al., 2005 and Samuelsson et al., 2006).

Also, Dhayanithi et al. (2010) reported that the aeromonas hydrophila considered as one of the most important stress related diseases that causes a great losses with a high mortality among fish.

So this study was planned to study the incidences of aeromonas hydrophils among O. niloticus irs risk symptoms and PM lesions and the methods of laboratory diagnosis with studying its economic losses in cultured oreochromis niloticus.

II. MATERIALS AND MEDTHODS

Bacterial isolation:

Bacterial isolation was attempted from the open ulcerative lesions in skin, epidermal hemorrhagic patches, spleen, liver , kidneys and heart blood of 390 moribund fishes.

Blood agar (5 % sheep blood), brain heart infusion agar and R.S. media were used for primary isolation. Smears from spleen and blood were stained with Giemsa's stain. The biochemical characteristics of the isolated bacteria were studied.

Using API-20 E system. Identification of the isolates was carried out according to Bergey's Manual of Determinative Bacteriology ***(Buchanan and Gibbons, 1974)*** and ***Whitman (2004).*** The antibiogram of the isolates was conducted on sensitivity agar (Muller hinton agar) according to the method mentioned by ***Lanyi (1980).***

Experimental infection:-

The isolate (5) of ***A. hydrophila*** incubated at 32 ^{o}C for 18 hours was used in experimental infection of healthy 1 year old ***O. niloticus*** (280 ± 10 g , 25 ± 2 Cm) obtained from Barsek Fish Farm. A group of 10 fish was injected with 0.5 ml / fish intraperitoneally from suspension containing 3 X 10^5 bacterial

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cells / ml as counted using plate count agar medium, (Oxoid). Further 10 fishes were kept 1 hour in water containing 3 X 10^5 bacterial cells/ml. The infected fish were kept in 60 L aereated aquaria supplied with tap water. Re-isolation of the organism was tried from the internal organs of dead and moribund fishes.

The medium lethal dose (LD_{50}) was calculated in (Table, 4) following intramuscular inoculation of 0.1 ml of 18 hours old broth culture of isolate (5) in its second subculture / fish using 5 fish / dilution (10^{-1} – 10^{-8})according to the method described by ***Reed and Munech (1938)***

Histopathological examination:-

For histopathological studies, skin and underlying muscles, gills, spleen, liver and trunk kidney of infected fish were fixed in 10% formalin saline, embedded in Paraffin and stained with Hematoxylin and Eosin (H & E) ***(Hibiya, 1982).***

Economic losses:-

The economic losses of the fish due to exposure to enterococci were determined from dead fish, weight of dead fish and the losses in return due to dead fish according to the following equations *(*Atallah and El-Banna, 2005):

a- Weight of dead fish = Number of dead fish X Average weight of the fish (gm).

b- Losses in returns (L.E) = Weight of the fish (Kg) X Price of Kg fish (L.E).

Statistical analysis :

The incidences of enterococci among fish, incidence of dead fish was statistically analysed using Chi^2-test according to (SAS, 2004).

RESULTS

Clinical studies:-

During December 2008, January and February 2009, 65 % mortalities (390 fishes out of 600) among broad stock of ***O. niloticus*** (2 years old; 2 – 3 Kg in weight : 45 ± 5 Cm in length) were observed wintering. More than 80 % of the ***O. niloticus*** showed hemorrhagic patches allover the body particularly around the base of the fins sometimes darking of the skin. In some moribund fishes, necrotic (frayed) fins, hemorrhaged scale pockets and pale pockets and pale gills indicative of anemia (Fig. 1,2 and 3) as well as edematous musculature . Internally, organs are friable and have a generalized hyperemic appearance; the kidney and spleen are swollen; and the liver is often mottled with hemorrhage increased with light areas. The enlarged abdomen with ascitis. The body cavity contain a clear fluid but more often the fluid is bloody and cloudy. The intestine is flaccid, hyperemic, contains yellowish mucus, and is void of food . Blood, heart, kidney and spleen smears stained with Giemsa showed numerous number of rod shaped organism.

Results of bacteriological; examination :-

From the blood and internal organs of 87 fish showing typical symptoms, 20 bacterial isolates were recovered in pure culture. These isolates formed yellow-brownish, smooth, mucoid colonies after 24 hours on the used media (Table, 1). Further characteristics of the isolates are given in (Table, 2). Results of antibiogram are shown in (Table, 3).

Results of experimental infection :-

Experimental infection with ***A. hydrophilla*** resulted in death of all fish inoculated intraperitoneally in a period of 11 days. Moribund fish swam slowly, their body became dark in association with hemorrhages that were recognized in the kidneys, spleen, liver and gills. In the smears of the organs, the same capsulated bacteria were demonstrated by Giemsa stain. Re-isolation of the same bacteria was succeeded.

Table 1. Incidence of ***A. hydrophilla*** isolated from the blood and some internal organs of ***O. niloticus*** :

Strains	Organs						
	Blood	Skin lesion	Spleen	Liver	Kidney	Heart	Gills
A. hydrophilla	8	3	4	3	3	7	8

Table 2. Showing biochemical identification of ***A. hydrophilla*** (Biovar I, II, III, IV, V) ***(Whitman, 2004)***.

Shape	*A. hydrophilla*		*A. hydrophilla*
	Gram –ve, rods	A donitol	-
Oxidative/fermentative	-	Amygdalin	-
Cytochrome oxidase	+	Arabinose	+
Catalse	-	Caprate	+
Citrate, Simmons	+	Cellobiose	-
Gelatinase	+	Dextrose	-
SIM Sulphide	-	Dulcitol	-
Indole	-	Fructose	-
Motility	- +	Galactose	-
VP	-	Gluconate	+
H2S	-	Glucose	+
Indole (Peptone H2O)	-	Glycerol	--
Nitrate reduction	-	Inositol	-
Tryptonase	-	Lactose	-
ONPG	-	Malate	+
Arginine dihdrolase	+	Maltose	V
Lysine decarboxylase	-	Mannitol	+
Ornithine Decarboxylase	-	Mannose	+
Esculin hydrolysis	-	Melibiose	-
Beta-galactosidase	-	Raffinose	-
N-Acetyl-Dglucosamine	+	Rhamnose	-
Phenyl-acetatae	-	Salicin	-
Triple sugar iron	-	Sorbitol	-
Urease	+	Sucrose	-
O/129 disk	-	Trehalose	-
TDA	-	Xylose	-
Adipate	-		-

+, 90 % or more of strains are positive -, 90 % or more of strains are negative

V, Strain variability * Motile by Polar flagella

Studies on the **LD_{50}** of isolate (5) indicates its high virulence to all of the tested ***O. niloticus*** (Table, 4) . The sensitivity test occur to ***A. hydrophilla*** strain No. 5 only which used for experiment .

Table 3. Results of antibiogram of ***A. hydrophilla***.

Antibiotic	Susceptibility	Antibiotic	Susceptibility
Penicillin	R	Nitrofuration	R
Chloramphenicol	R	Chlorotetracyclin	s
Streptomycin	s	Oxytetracycline	s
Tetracyclin	s	Kanamycin	S
Neomycin	S	Gentamycin	S
Polymyxin B	s	Novobiocin	R
Erythromycin	R	Spiramycin	R

R= Resistant S= Highly sensiti s= Moderately sensitive

Table 4. LD_{50}-value of ***A. hydrophilla*** (Isolate 5) in different fishes by intramuscular inoculation.

Fish species	LD_{50}-value
Oreochromis niloticus, (25 g ± 5; 10 Cm ± 2)	4.8 X 10^7

N.B. Length measurements are those of total length.

Results of histopathological examination :

Examination of stained sections of skin and internal organs of naturally and experimentally infected O. niloticus revealed the following histological alterations; dilatation of the dermal and hypodermal blood vessels associated with focal areas of haemorrhages.

Perivascular aggregation of melanomacrophages could be recognized. Oedematous fluid was seen between the swollen dermal collagen fibers (Fig. 4). Congestion of the blood vessels was markedly seen in the liver, kidney and splenic sinusoids (Fig. 5). Excessive number of immature erythrocytes was between the renal tubules. Homogenous eosinophilic proteinous substance was seen in the renal tubules together with intertubular haemorrahes (Fig. 4, 5). Submucosa of blood vessels of intestine dilated together with focal areas of haemorrhages and leucocytic aggregation were seen.

Treatment was successful, and mortalities ceased 6 days following the application of the last dose of the antibiotic.

Mortality pattern and economic losses :

Table (5) cleared that, the mortality percentage due to aeromonas hydrophila reached to 65 % from the examined fish in studied farms. And the weight losses due to aeromonas hydrophila reached to 162.50 Kg of fish sale, while, the the return losses reached to 1625 LE/100 fish suffering from infection with aeromonas hydrophila.

Table (5) : Mortality rate and economic losses in aperiod of 11 days after bacterial inoculation intraperitoneally.

Parameter	A group of 10 fish was injected with 0.5 ml / fish intraperitoneally from suspension containing 3 X 10^5 bacterial cells / ml as counted using plate count agar medium, (Oxoid).
Total number of fish examined	600
Mortality due to A. hydrophila	390
Average wt of the fish	2.5 Kg
Percentage of death	65 %
Wt losses / 100 fish	65 X 2.5 Kg = 162.50
Price of Kg fish	10
Return losses / LE/100 fish	1625

III. Discussion

Since plehn (1904) reported for the first time ***A. hydrophilla***. as dangerous for the O. niloticus, reports confirming the high threat of this bacterium to cultured fish were published in an uninterrupted manner ***(Richards et al., 1985).*** Then researches are continue untile the ***(Wakabayashi and Egusa, 1972)*** who first recognized the ***A. hydrophilla*** in pond cultured eels (Anguilla japonica) which induce Red Spot Disease. In ***1987 Fasial and Easa*** found that the unidentified ***Aeromonas Spp.*** Infected tilapia under stress condition during transporation from Hungary to Cairo via aeroplans and then from Cairo the Behera. Among other fish pathogenic ***A. hydrophilla***, which was reported to induce high mortalities among common carp ***(Heuschmann-Brunner, 1987);*** grass carp ***(Lobuncov, 1972);*** tench ***(Ahne et al., 1982)*** and tilapia frys ***(Duremdez and Lio-Po, 1985).***

The high susceptibility of ***O. niloticus*** to experimental infection with different ***A. hydrophilla*** Isolated from other fish species was reported by ***Amin (1974)*** identified several ***A. hydrophilla*** from the intestinal contents of healthy O. niloticus. The same author reported also the isolation of ***A. hydrophilla*** from the Parenchymatous organs of apparently healthy fish species.

The isolates (5) of ***A. hydrophilla*** recovered during the course of this study, resemble in its morphological and biochemical characteristics these of ***A. hydrophilla*** isolated by Bullock (1965) from the cyprinid *Carassius aurata*.

Experimental infection was successful only when the organism was injected and not when the fishes were left in infected water. The ***A. hydrophilla*** injected intraperitoneally (ip) by dose 0.5 ml of suspension contain 3 X 105 bacterial cells/ml. This indicates that, the penetration of the bacteria is a precondition of infection ***(Austin and Austin, 1987; Faisal and Easa 1987 and Plumb, 1994).***

These suggest that erosions, injuries during handling and different stressors play an important role in the pathogenesis of the disease. This outbreak was preceeded with a long transport which had been done as perfect as possible. In comparison to other reported ***A. hydrophilla*** -caused outbreaks, the mortality rate observed in this study is considered relatively similar. In one experiment, ***Ahen et al. (1982)*** achieved 100 % mortality in tench fry within 10 days at a water temperature of 10 oC. Challenge was by intraperitoneal injection of a bacterial suspension. This may be attributed to the high susceptible of ***O. niloticus*** from one side and to the bad handling, low temperature and transport from the other side.

Results of LD50-values indicate a prime pathogenicity of ***A. hydrophilla to O. niloticus.*** This benefits in as the ***A. hydrophilla*** may constitute a hazard particularly in polyculture fish farms, where ***O. niloticus*** is stocked with other fish species to achieve a maximum utilization of the primary and secondary productivity ***(Hass, 1982).***

Histological examination revealed a typical septicemia picture with dilatation of the dermal blood vessels associated with focal areas of haemorrhages in addition to congestion of the blood vessels seen in the liver, kidney and splenic sinusoids. This picture was described by ***Li and Jordan (1968)*** and ***Wakabayashi and Egusa (1972)*** on both ***Rainbow trout (Salmo gairdneri) and Japanese eels (Anguilla japonica)*** infected with ***A. hydrophilla .***

Ahne et al. (1982) and Faisal and Easa (1987) attributed the pathogenic effect of ***A. hydrophilla*** to the production of extracellular proteinase enzyme which attach the endothelial lining of the blood vessels and any cells in the musculature and paranchymatous organs. In this respect, it is worthy to mention that haemorrhagic patches developed in different organs and generalized hyperemia in kidney, spleen and liver of ***O.***

niloticus may be attributed to the influence of the bacterial toxins.

Within several days of being injected with ***A. hydrophilla, O. niloticus*** become moribund and show clinical signs similar to naturally infected fish with mortality occurring in 2 to 11 days where all fish die. Medicated feed containing effective results found that the neomycin, kanamucin, gentamycin and oxytetracycline were the most common antibacterial drugs stopped mortality after used for 3 consecuative days resulted in 100 % survival. On other side the resistance of our ***A. hydrophilla*** to most antimicrobial drugs complicates the chemotherapy in such outbreaks. ***(Bejerano et al., 1979; Faisal & Easa 1987 and Weile et al., 2007).***

Our results in economic losses cleared that, the aeromonas hydrophila causes a great economic losses to the fish farms. The economic losses of aeromonas hydrophila attributed to the higher mortality rate of the fish that causing increasing losses in body weight sale and decreasing of the economic returns. As our results cleared that, the mortality rate due to infection of the O. niloticus with aeromonas hydrophila reached to 65 % that causes a great losses in the weight mass sold fish reached to 162.5 Kg/100 fish with a high economic losses in fish returns that reached to 1625 LE/100 fish.

Our results agreed with those of ***(Klesius et al., 1997*** and ***Locke et al., 2007),*** where they reported that, the aeromonas hydrophila causes a high mortality to the fish farms with increasing to the body weight losses and increasing the return losses.

The results of this study indicated that, the aeromonas hydrophila causes a great economic losses with a high mortality to areochromis niloticus cultured fish production farms and the rapied laboratory diagnosis with rapied treatment is the main methods for controlling its spreading.

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Fig. 1. Oreochromis niloticus naturally infected with A. hydrophila showing congestion of all body surface and tail rot.

Fig. 2. Naturally infected Oreochromis niloticus with A. hydrophila showing ulceration of the head region and festula formation.

Fig. 3. Naturally infected Oreochromis niloticus with A. hydrophila showing hemorrhage and congestion of the gills and liver and gall bladder with generalized septicemia .

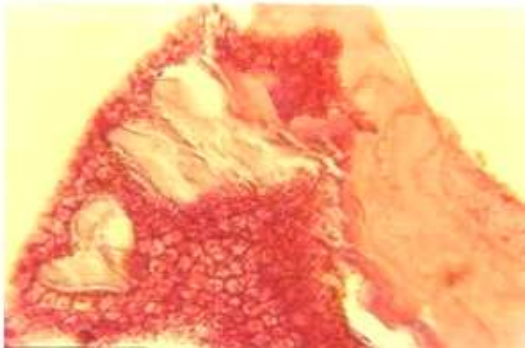

Fig. 4. Skin of Oreochromis niloticus infected with A. hydrophila showing : epidermal hyperplasia, necrosis and sloughing made ulceration containing pale eosinophilic mucoid material. (H & E, X 400) .

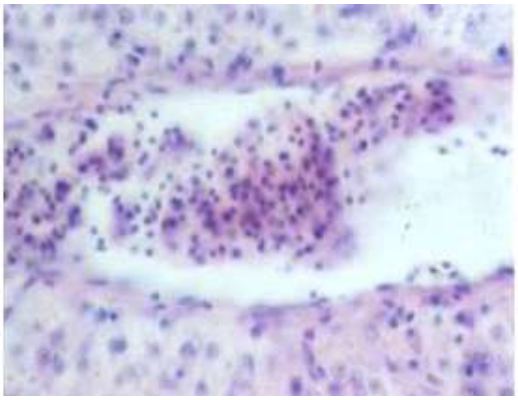

Fig. 5. Liver of infected Oreochromis niloticus with A. hydrophila showing congestion of blood vessels . (H & E x 150) .