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CONTRIBUTION TO MOTILE AEROMONAS SEPTICAEMIA IN SOME CULTURED AND WILD FRESHWATER FISHES

By

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SUMMARY

This study was carried out to investigate more information about the prevalence of Motile Aeromonas Septicaemia (MAS) in both cultured and wild Nile tilapia and Karmout catfish. The clinical signs and P.M. lesions were pronounced in cultured fishes. *Aeromonas hydrophila* was recovered from all MAS affected fishes. The incidence of MAS in cultured and wild Nile tilapia was 10.0% and 2.5% respectively while it was 18.75% and 6.25% in cultured and wild Karmout catfish respectively. Reisolation of *A. hydrophila* was recovered after experimental infection of Karmout catfish by I/P and S/S revealing the same clinical signs and lesions. The histopathological pictures produced by such microorganisms along the course of experimental infection were discussed. Besides, *A. hydrophila* isolated from cultured fishes were more resistant to antibiotic sensitivity test than those isoalted from wild fishes.

INTRODUCTION

Among bacterial fish diseases, Motile Aeromonas Septicaemia (MAS) has been reported to cause serious epizootics with extensive economical losses in freshwater and marine fishes (Gatti and Nigrelli, 1984; Rahim and Kay, 1988; Ferguson, 1989; Eissa et al., 1991, Leung et al., 1992 and Eissa et al., 1993). Besides, motile aeromonads pose a dangerous threat as pathogens of human beings; they cause enteritis, dermatitis and encephalomyelitis (Geneviene et al., 1970; Ketover et al., 1973; Atkinson and Trust, 1980 and Stospskof, 1993).

The present study was planned to focus a light on this problem in both wild and cultured freshwater fishes (Nile tilapia, *Oreochromis niloticus* and Karmout catfish, *Clarias lazera)*, through incidence, clinical signs, postmortem lesions, experimental infection, antibiotic sensitivity test of the isolated bacteria, besides the study of histopathological alterations.

MATERIAL AND METHODS

A total of 160 fishes of which (80) Nile tilapia (*O. niloticus)* and (80) Karmout catfish (*C. lazera)* were caught at random samples from Fish Hatcharies at El-Abbasa Fish farm as cultured fishes. The same number 160 fishes from hte above two mentioned species, distributed as (80) for each one and obtained from Nile tributaries in Ismailia as wild fishes.

Both cultured and wild fishes were subjected to full clinical and postmortem examination according to the methods given by Amlacher (1970).

Samples for bacterial examination from fishes showing signs of septicaemia were taken and cultivated on Nutrient agar, MacConkey's agar, Brain Heart Infusion agar, Trypticase Soy agar and Rimler and Shotts agar. The plates were incubated at 25°C for 24 hrs. Suspected pure colonies of *Aeromonas hydrophila* were transfered into nutrient slants for further identification using the morphological characters, colonial and growth appearance and biochemical tests according to Krieg and Holt (1984).

Experimental infection was carried out using 80 live and apparently healthy fishes (40 for each species), with an average body weight 80 ± 5g. Each fish species (EFS) was divided into 4 equal gorups each of 10 fish. The 1st group of EFS was inoculated intrapertonelay with a dose of 0.5ml of 24 hrs old broth culture of *A. hydrophila* (3 x 10/ ml living bact. cells), while the 2nd group of EFS was dipped in the cultured broth for 5 minutes after skin scarification (Lucky, 1977). While, the 3rd and 4th groups of EFS were served as control groups and injected I/P and S/S with o.5 ml sterile broth. All experimentally infected fish were observed daily for two weeks for any clinical signs and mortalities. P.M. examination was done and bacterial re-isolation fo *A. hydrophila* was attempted.

Specimens for histopathological examination were taken from liver, intestine and kidneys of experimentally infected catfish *Clarias lazera*. They were preserved in 10% neutral buffer formalin, dehydrated, embeded in parafin, sectioned at 4-5µ and stained with H and E (Drury and Wallington, 1980).

Antibiogram of recovered *A. hydrophila* isolates from both wild and cultured fishes were done using the method of Fuhrmann (1983). The interperitation of zones of inhibition were estimated according to the limits given by Bio-Merrieux (1984)

RESULTS

The clinical examination of naturally affected c tured and wild Nile tilapia and Karmout catfish w MAS revealed generalized erythema, congested f dark discolouration of the skin 'exophthalmia a pronounced abdominal distention (ascitis). Th signs were more clear in cultured fishes. Fig. 1, 2 a 3.

P.M. findings showed congestion in the internal gans, petechial haemorrhages in the peritoneum a most of the visceral organs as well as erythema in intestine with fishes. bloody fluid. These lesi were more pronounced in cultured fishes. Fif. 3 a 4.

The bacterial examination revealed that the isola bacterial pathogens were Gram-negative, short r tile bacili. The isolates gave positive results with dole test, oxidase test, gelatin hydrolysis and Vo Proskour while were negative with methyl red, u hydrolysis and H_2S production. The isolates g acid with glucose, sucrose, maltose and salicine. T isolates were identified as *A. hydrophila*.

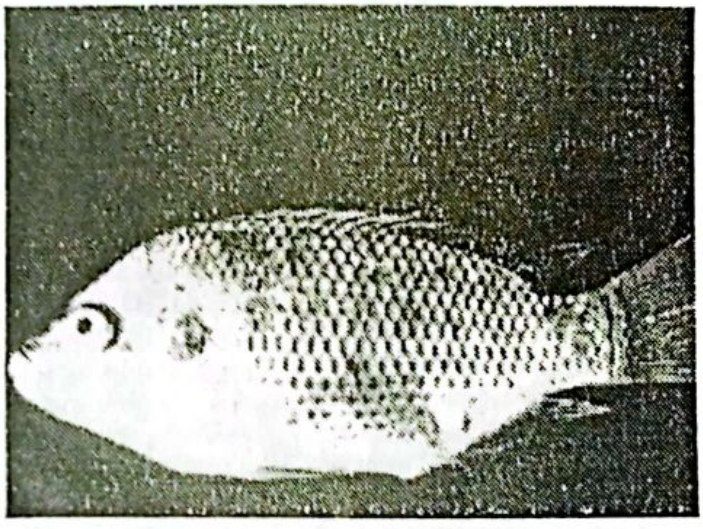

Fig. (1): A Nile tilapia fish showing erythema, protruded vent and exophtholmia.

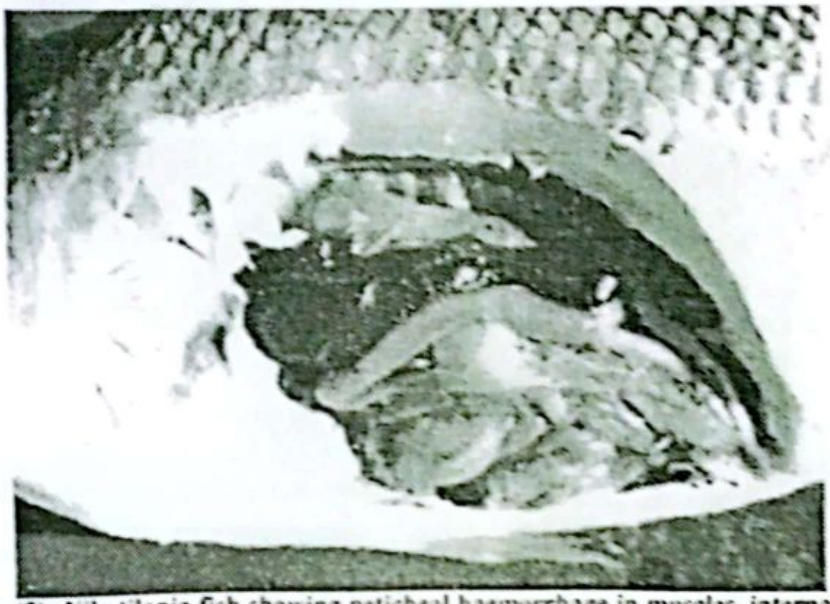

Fig. (2): Nile tilapia fish showing peticheal haemorrhage in muscles, internal organs and intestine filled with mucus.

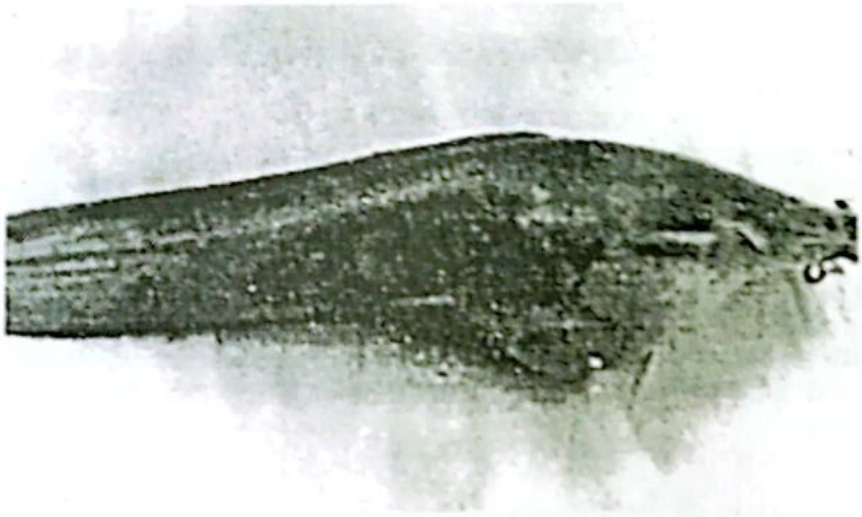

Fig. (3): Karmout catfish showing abdominal distention (ascitis).

Table (1): Incidence of MAS in cultured and wild Nile tilapia and Karmout Catfish.

Fishes	Total No.	No. of diseased fishes	%	No. of MAS diseased fishes	%	% of MAS to Total No.
Cultured	80	23	28.75	8/23	34.78	10.00
Nile tilapia	80	9	11.25	2/9	22.22	2.50
Wild	80	32	40.00	15/32	46.87	18.75
Cultured						
Karmout catfish	80	14	17.50	5/14	35.71	6.25
Wild						

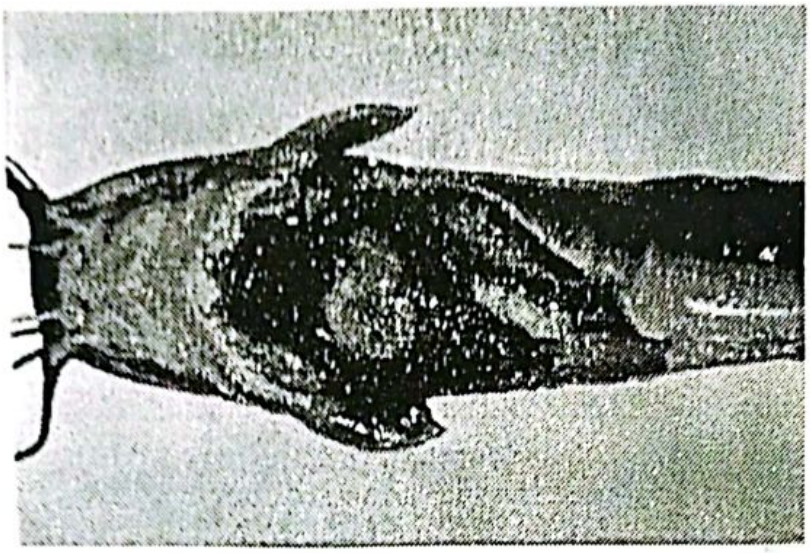

Fig. (4): Karmout catfish showing general congesion of internal organs and haemorrhagic muscles.

Table 1 shows the incidence of MAS in cultured (C) and wild (W) Nile tilapia and Karmout catfish. It reveals the percentage of total diseased fish to the total number of examined fishes as 28.75% (C); 11.25% (W) and 40.00% (C); 17.50% (W) respectively in both fishes. The percentage of MAS diseased fish to total diseased fishes were 34.78% (C); 22,22% (W) and 46.87% (C); 35.71% (W) in both fishes respectively. While, the percentage of diseased fish with MAS to the total number of examined fishes were 10.00% (C); 2.50% (W) and 18.75% (C); 6.25%(W) in both fishes respectively.

Table (2) reveals the results of experimental infection with *A. hydrophila* by I/P and S/S routes in Nile tilapia and Karmout catfish. The mortality rate was 60% and 80% in Nile tilapia with I/P and S/S respectivley, while it was 70% and 100% in Karmout catfish by the same routes respectively. The experimental infected fishes showed the same clinical signs and P.M. lesions observed in natural

Table (2): Experimental infection with *A.hydrophila* by different routes in Nile tilapia and Karmout catfish.

Fishes	Route of inoculation	Challanged fish *			control fish**			Temperature
		No.	MR.	%	No.	MR.	%	
Nile tilapia	I/P	10	6/10	60	10	0/10	0.0	25° ± IC
	S/S	10	8/10	80	10	0/10	0.0	
Karmout catfish	I/P	10	7/10	70	10	0/10	0.0	
	S/S	10	10/10	100	10	0/10	0.0	25° ± IC

* Dose = 0.5 ml $3x10^7$
** Dose = 0.5 ml sterile broth

infection indicating MAS. The injected *A. hydrophila* was re-isolated again from all freshly dead and clinically diseased fishes.

Histopathological changes induced by experimental infection with *A. hydrophila* in Karmout catfish were shown in Fig. 5 and 6 There were vacular degeneration with large and rounded vacules with degenerative changes in the hepatocytes and hyperplastic activation of Kaupffer cells in the liver. Kidney showed slight diffuse suppurative nephritis, vacular degeneration of the renal tubules, congestion of blood vessels and diffuse infiltration with heterophils in the interstitial tissue and glomeruli. Intestine showed catarrhal enteritis, leukocytic infilteration of lamina propria and oedema in all gut layers.

Concerning the sensitivity of *A. hydrophila* strains of both cultured and wild fishes to different anti-

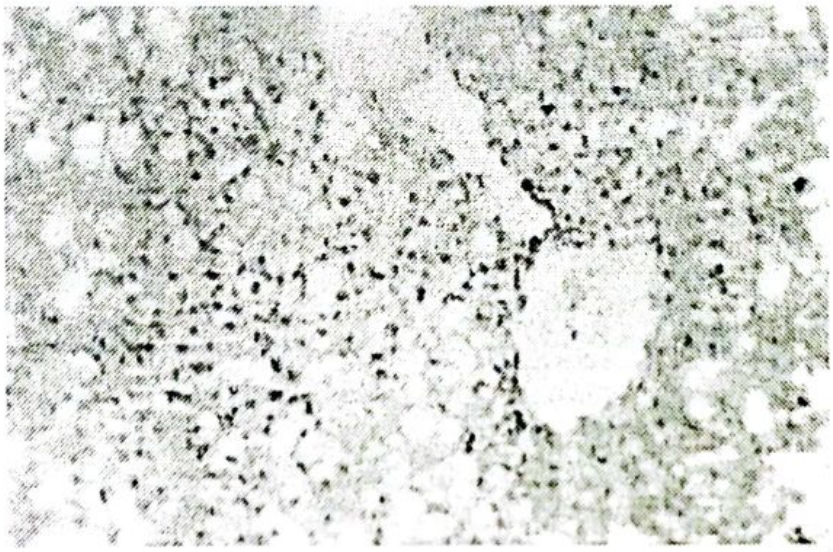

Fig. (5): Liver of Karmout Catfish showing large and round vacular degeneration of hepatocytes pushing the nucleii to one side. H & E (X 300).

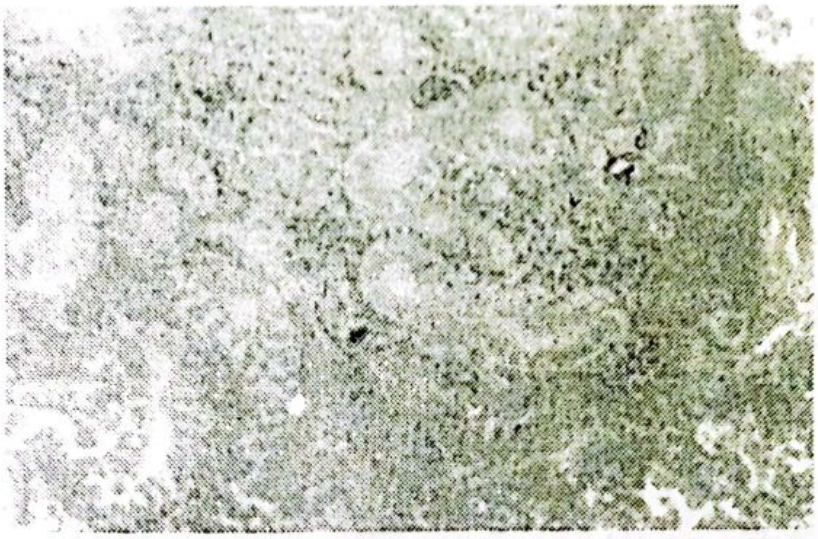

Fig. (6): Kidney of Karmout Catfish showing activation of haemopoetic tissue and congesion of blood vesseles. H & E (X 300).

Table (3): Antibiotic senstivity of recovered *A hydrophila* from wild and cultured fishes

Antibiotics / isolate	Gentamycin	Chloram phincol	Erythro mycin	Ampicil lin	Oxytetr acyclin	Lincom ycin	Neomycin
From cultured fish	R	S++	R	R	S+	R	R
From wild fish	S	S++	R	R	S+++	R	S+

+++ = high sensitive ++ moderate + = low sensitive R= resistant

biotics. The cultured strains were sensitive to chloramphinicol and Oxytetracyclin while wild strains were sensitive to Gentamycin, Chloramphinicol, Oxytetracyclin and Neomycin (Table 3).

DISCUSSION

The present study revealed that cultured and wild fishes are affected by the same agents, but the incidence of epizootics is greater among intensively cultured than free living fish.

It was revealed that the clinical signs and P.M. lesions of naturally infected cultured Nile tilapias and Karmout catfish were prominent than ocured in the same fishes in wild state. These results were hand by hand with the high incidence found cultured Nile tilapia and Karmout catfish (10.00% and 18.75%) while in those fishes in wildness were (2.50% and 6.25%) As intensive culture fish farming is the most economic and widely spread method, yet fishes grown by this method are exposed to various stressors mainly overcrowdness which leads to increase of organic matter and quick spread of infection especially with aquatic commensal bacteriae as *A. hydrophila* (Plumb, 1984; Kumar and Dey, 1988 and Eissa, et al., 1991). This explains why incidence of MAS in cultured fishes is greater than wild ones. Also, the variations in incidence of MAS in both fishes may be due to the difference in susceptiblity of Nile tilapias which had lower incidence than karmout catfish. Nile tilapias has high resistance as they are well armoured scaley fish while Karmout catfish are bottom feeders and scaleless fishes, so they are more exposed to infection (Odum, 1970 and Shepherd and Bromage, 1988).

Regarding the experimental infection, the ... route of *A. hydrophila* in both fishes was more ... fective in producing mortality and lesions than ... route (Table 2). It could be attributed to that ... *hydrophila* can enter susceptible fishes thro... external injuries (Post, 1987).

A. hydrophila could be identified by the cultu... morphological and biochemical tests. These fi... ings agree with those of Shotts and Rimler (19... Ewing et al. (1981); Krieg and Holt (1984) a... Eissa et al. (1991). The pathogenicity of *A. h... drophila* to fish was attributed to the production ... proteolytic enzymes (Kou, 1972) and produc... of extracellular enzymes, cytotoxins and haemo... sins (Wakabayashi et al., 1981).

Regarding the histopathological alterations in ... experimentally infected karmout catfish with ... *hydrophila* revealed cular degeneration, degen... ative changes in the hepatocytes and hyperplas... activaition of Koupffer cells. The kidney show... diffuse suppurative nephritis, vacular degen... tion of renal tubules and congestion of blood v... sels. The intestine showed catarrhal enteritis, l... kocytic infiltration of lamina propria and oede... in all gut layers. These changes were also ... served by Huizinga et al. (1979) and Ventura ... Grizzle (1988).

The antibiotic sensitivity test revealed that *A. ... drophila* isolated from cultured fishes was m... erately sensitive in vitro to chloramphenicol ... weak sensitive to oxytetracyclin, while it was ... sistant to gentamycin, erythromycin, ampi... lin,neomycin and lincomycin. In the other side, ... *hydrophila* isolated from wild fishes was hig... sensitive to oxytetracyclin and moderately se...

tive to gentamycin and chloramphenicol and weakly sensitive to neomycin. From the available literature, there are no studies dealing with the comparison of sensitivity of *A. hydrophila* isoalted from cultured and wild fishes. The present study suggests that *A. hydrophila* isolated from cultured fish was more resistant than those isoalted from wild fishes due to the possibility of previous treatment with antibiotics. It was concluded that cultured fishes must be checked routinely with proper fish management and suitable treatment as they are less resistant than wild fishes.

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