Role of Freshwater Crayfish *Procambarus clarkii* in Transmission of Motile Aeromonas Septicemia to Nile Tilapia *Oreochromis n*(one mark)

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Abstract
A total of 60 Nile tilapia fish *Oreochromis niloticus* were collected from River Nile (Damietta branch): Weesh El-Hagar village during late summer. An experimental infection was applied on apparently healthy Nile tilapia (three equal groups) by intramuscular injection of 0.1 ml of $2.4X10^7$ CFU/ml of well identified *A. hydrophila* suspension isolated from freshwater crayfish *P. clarkii*, while, the third group was injected 0.1 ml of sterile buffered saline . The clinical picture, morbidity and mortality rates were recorded for 7 days. The results showed that all injected tilapia died by the end of experiment with a mortality peak on the 3rd day, while the morbidity started to appear by the 2nd day (10%). The histopathological alterations were recorded and discussed. This study suggests an epidemiological significance of *Aeromonas hydrophila* and the role of crayfish in its transmission.
Keywords: *Oreochrmis niloticus*, *Procambarus clarkii, Aeromonas hydrophila*

Introduction

Procambarus clarkii is considered the most important crayfish known in the world and has been successfully introduced to all countries. *P. clarkii* seems to have been introduced recently in Egypt (***Fishar, 2006)***. Within the last few years, it has been established in various sites of the River Nile and its branches ***(Hamdi, 1994)***. Many bacterial pathogens could infect fish in different virulence degrees ***(Ostfeld et al, 2008)***. Unbalanced conditions: malnutrition, poor water quality, overstocking or other stressors of fish control pathogenicity of bacteria ***(Lipp and Ross, 1997** and **Xu et al, 2012a)***.

Motile Aeromonas Septicemia (MAS) is the most common bacterial disease of freshwater

fishes. This disease has been associated with several members of the genus Aeromonas, including *Aeromonas hydrophila, A sobria, A. caviae, A schuberti,* and *A. veronii.* Moreover, the role of *A. hydrophila* as an etiological agent in fish and shellfish diseases is highly significant ***(Marcel et al, 2013)***. The current study was aimed to investigate the role of infected *P. clarkii* with *A hdyrophila* in transmission of MAS to *O. niloticus* through its challenge experimentally.

Materials and Methods:

Fish:

A total of 60 *Oreochromis niloticus* were collected from River Nile (Damietta branch): Weesh El-Hagar village Mansoura, Dakahlia governorate during late summer, with an average body weight 90±10 g. Fish were acclimated in fully prepared glass aquaria (80 X40 X60 cm.) for one week and then directed for experimental test.

Bacterial isolate:

A well identified isolate of *A. hydrophila* (kindly obtained from Dept. of Fish Diseases and Management, Fac. of Vet. Medicine, Suez Canal Univ). That isolated from clinically diseased *P. clarkii* and identified by traditional biochemical and molecular methods.

Preparation of the bacterial isolate:

A. hydrophila strain was inoculated onto Tryptic Soy Agar (TSA) plates and incubated at 28°C for 24 hours. The bacterial cells were collected in 5 ml phosphate buffered saline (PBS) and adjusted to 2.4×10^7 CFU/ml using MacFerland tubes according to ***McFarland (1970)***

Pathogenicity test

Sixty fish were divided equally into three groups: 1 and 2, while group 3 was kept as control. 0.1 ml of 2.4×10^7 CFU/ml of *A. hydrophila* suspension was intramuscularly injected into each fish using 21 gauge sterile needles within the two replicate groups. The control group was injected with 0.1 ml of sterile buffered saline. The observation time was 7 days. Clinical pictures as well as numbers of dead and infected fish were recorded daily to calculate the morbidity and mortality rates according to ***Al-Dughaym (2000)***.

Reisolation and identification:

The bacterial strains were re-isolated from dead and moribund fish, then examined under complete aseptic conditions. Swabs from liver, spleen, kidneys, gills and musculature of Nile tilapia were taken for further bacteriological and histopathological examinations. The collected swabs were smeared separately onto tryptic soya agar and incubated at 30 ºC for 24hr., then streaked directly on Aeromonas specific media (RAYAN). A loopful from each pure culture was inoculated into a slant of nutrient agar for further biochemical identification of isolates and into another semi-solid

nutrient agar. Identification of isolated bacteria was carried out using microscopic examination and cultural characters according to schemes of biochemical reaction according to ***Cruickshank et al (1982)***.

Histopathological examination:

Tissue specimens about 0.5cm in thickness were collected from gills, kidneys, liver, spleen and musculature. The collected specimens were rapidly fixed in 10% neutral buffered formalin. The fixed specimens were processed for histopathological technique. Five microns paraffin sections were prepared and stained with haematoxalin and eosin for microscopical examination according to **Takashima and Hibiy (1995)**

Results:

Isolation and identification:

Isolated *A. hydrophila* was of dark green, opaque with darker center colonies on RAYAN. Besides, cultural, morphological and biochemical characters.

Experimental infection:

All experimentally challenged fish with *A. hydrophila* died within 7 days. The peak of mortality was observed on the 3rd day of experiment (32.5%), while the morbidity started to appear at the end of 2nd day (10%) table (1).

Clinical picture of experimentally infected Nile tilapia:

Plate (1) showed that the clinical findings that began to appear at the end of the 2nd day of experimental infection, where some of the injected fish were stagnant. On the 3rd day, heamorrahge of caudal trunk associated with small ulcers under dorsal fin were noticed, while on the 4th day focal heamorrahge, ascites associated with partial desquamation of scales and mild congestion of liver and spleen were also found. By the 5th day fish became reluctance to eat associated with heamorrahge and erosion of caudal fin, opaque eye, ulceration of caudal peduncle and sunken eye**.** By the 6th day, fish were swimming closer to surface and aquarium wall, off food, unstable swimming on the bottom of the aquarium. By the 7th day only 2 fish still alive to show pale gills and progressing skin ulceration.

Histopathological findings:

Plate (2: a) showed gills of *O. niloticus* exhibited shorten and denuded secondary lamellae appeared with large pale chloride cells while few macrophage infiltration found in secondary lamellae together with abundant lymphocytes. Slight edema was recorded in between secondary lamellae. Massed of nodular proliferation like were seen especially at the free portion of some secondary gill filament. Some secondary lamellae appeared as very thin filaments with necrotized epithelium. Masses of cells appeared in between the secondary lamellae composed of lymphocytes, blood cells and little

desquamated epithelial cells. Gill fusion with club formation and edema in between secondary lamellae was recorded. In Plate (2: b) liver showed vacuolation associated with congestion and dilatation of portal blood vessels: central veins and hepatic sinusoids, while plate (2:c) showed disorganization of hepatic cords with tendency to adenoid formation and minute heamolytic areas appeared in between some heamocytes. Spleen of *O. niloticus* plate (2: d) showed severe depletion of lymphoid elements with activation of melanomacrophage centers in addition to severe congestion of splenic blood vessels. Destruction of some parts of epithelial lining of splenic blood vessel with increasing size of melanomacrophage centers. In kidney plate 2: e and f), severe degeneration and necrosis of renal tubular epithelium were the common view in all fish. Cystic formation of some renal tubules was also recorded while some clusters formation was also seen cells in cell centers leaving its basement membrane. The glumeruli showed edema in their mesangial spaces, some of them showed hypercellularity, while the most common histopathological changes revealed glumerulonephosis. Finally, mausculature plate (2: g) showed disorganization of its bundles with zinker necrosis, while in plate (2: h), start of splitting appeared with edema and start of inflammatory reaction.

Table (1): *Showing morbidity and mortality rates of experimentally infected O. niloticus.*

Dose	Control group (20) 0.1 ml of sterile buffered saline				Injected fish (40) 0.1 ml of 2.4×10^7 CFU/ml of *A. hydrophila*			
Days	Morbidity		Mortality		Morbidity		Mortality	
	No.	%	No.	%	No.	%	No.	%
1st	0	0	0	0	0	0	9	22.5
2nd	0	0	0	0	4	10	8	20
3rd	0	0	0	0	6	15	13	32.5
4th	0	0	0	0	5	12.5	5	12.5
5th	0	0	0	0	1	2.5	1	2.5
6th	0	0	1	5	2	5	2	5
7th	0	0	0	0	2	5	2	5
Total	0	0	1	5	20	50	40	100

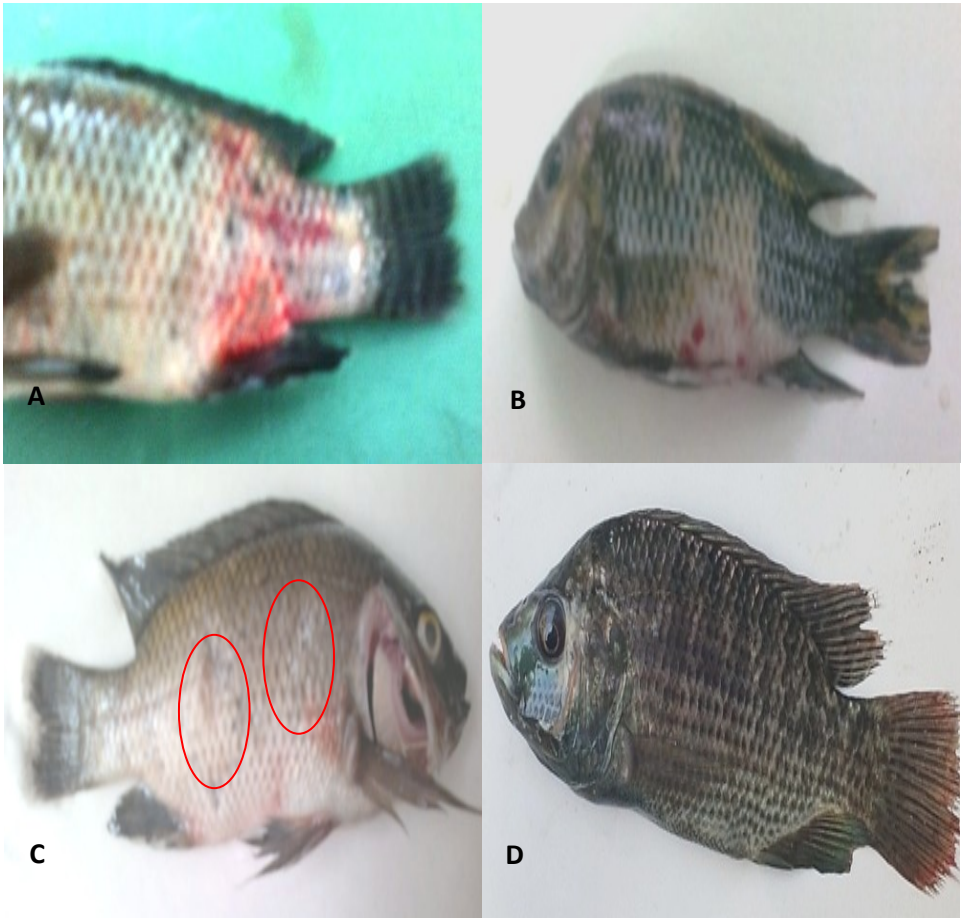

Plate (1) showing clinical picture of *O. niloticus* post injection with *A. hydrophila*
A: 3rd day post injection :heamorrahge of caudal trunk
B: 4th day post injection :ascetic, focal heamorrahges around vent and partial sloughing of scales
C: 6th day post injection: pale gills, progressing skin ulcers and tail fin rot
D: 7th day post injection: darkness of whole body and emaciation

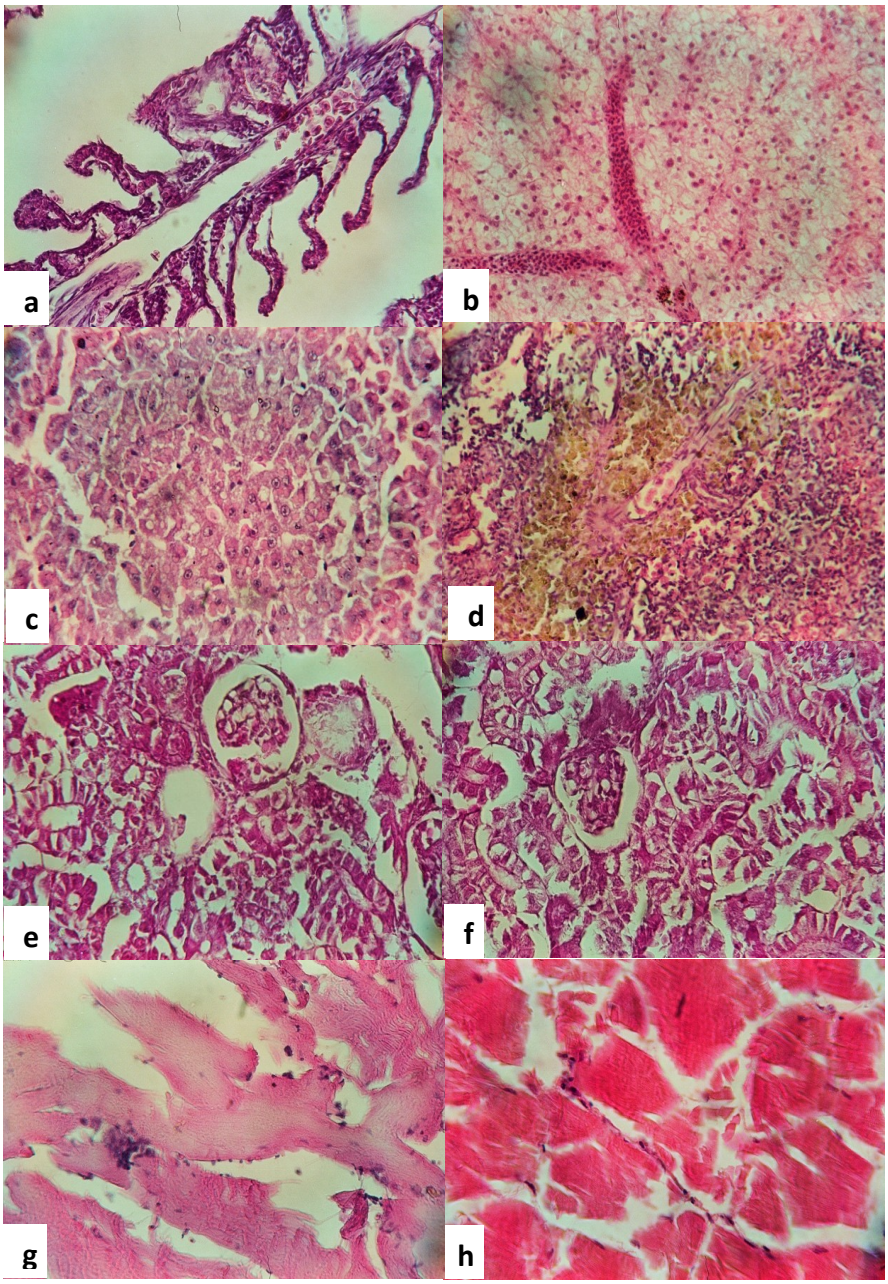

Plate (2): clinically diseased *Oreochromis niloticus* injected with suspension of *A. hydrophila*

a:Gill fusion with club formation and oedema in between secondary lamellae, H&E X400
b:liver congestion and dilatation of blood vessels. H&E X400
c:hepatic cord disorganization with adenoid formation. H&E X650
d:spleen showing diffuse melanomacrophage cells with haemocedrosis. H&E X400
e:kidney showing cystic formation with degeneration, necrosis and clusters of cells in its center. H&E X400

Discussion

The present study showed that all Nile tilapia experimentally infected with *A. hydrophila* (isolated from crayfish) died within 7 days. The peak of mortality was 32.5% on the 3rd day of experiment, while morbidity was 50% by the end of experiment. The moribund fish showed sluggish movements, swimming near the surface water, lethargic, roughness and easily detached scales, dark pigmented skin, hemorrhage at the base of the fins and sometimes losing of scales leaving ulcers, unilateral or bilateral exophthalmia, opaqueness of the eyes with hemorrhage and abdominal distention and finally loss of balance. This could be attributed to the lesions in the labyrinth and associated with maintenance of equilibrium, while the sluggish movement was probably due to the result of frayed tail and fins that agreed with those findings obtained by ***Abou El-Atta and El-Tantawy (2008)***. These results were not similar to that reported by **Ibrahem *et al. (2008)*** *and* ***Shayo et al (2012)****, while* ***Lio-Po et al (1998)*** reported *Aeromonas hydrophila* causing cumulative mortality of 50% in the experimental fish. These results may be attributed to the virulence degree of the bacterial strain isolated from infected crayfish.

Clinical picture began to appear at the end of the 2nd day of experimental infection, where some of the injected fish were stagnant. By the 3rd day heamorrahge of caudal trunk associated with small ulcer under dorsal fin were noticed. By the 4th day focal heamorrahge, ascetic associated with partial desquamation of scales mild congestion of liver and spleen were also found. By the 5th day fish became reluctance to eat associated with heamorrahge and ascetic of internal organs, erosion of caudal fin, opaque eye and ulceration of caudal peduncle. By the 6th day, fish were swimming closer to surface and aquarium wall and disappearance of eating, unstable swimming on the bottom of the aquarium. By the 7th day only 2 fish still alive to show pale gills and progressing skin ulceration and death occured. These findings were in agreement with ***Nielsen et al (2001), Ibrahem et al (2008)*** *and* ***Shayo et al (2012).*** The postmortem lesions of experimentaly infected tilapia were characterized by diffuse congestion of liver and spleen, liqufactive necrosis of liver with enlarged gall bladder, focal heamorrahgic liver, ascetic and heamorrahge of internal organs. A successful reisolation and identification of *A. hydrophila* from extra-intestinal organs of experimentaly infected tilapia.

These findings were nearly similar to that observed by ***Eissa et al (1994); Ali (1996)*** *and* ***Ibrahem et al (2008).***

Fish tissues revealed different degrees of pathological lesions such as telangectasis and club shape formation of secondary lamellae. This is a response to the branchial injury in which there is breakdown of vascuolar integrity due to the rupture of pillar cells and pooling of blood ***(Azad et al, 2001)***. Liver and kidneys are attacked by bacterial toxins that led to loose of their structure ***(Yousk and Napis, 2007)***. In addition, hepatic and splenic lesions were necrotic and degenerative changes with depletion of lymphoid follicles in spleen and increase heamocidrosis due to increase areas of melanomacrophage centers, these results were obtained also by ***Tsai and Liu (2006)***. The increase of heamosidrosis in spleen was attributed to β-heamolysin inside fish body that causing heamolysis followed by deposition of on Tilapia in Aquaculture.

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Ali M. (1996): "Studies of the pathological changes in *Tilapia nilotica* (*O. niloticus)* due to Motile Aeromonads infection". M.V. Sc. heamosidrin ***(Noga, 2010)***. Owing to fish musculature, zinker necrosis, hyalinization and inflammatory mononuclear cells and oedema between muscle bundles were recorded in this study may be contributed to *Aeromonas hydrophila* toxins, this also suggested by ***Azad et al (2001)***.

From the current study it could be concluded that, there may be a strong correlation between crayfish release during summer and the increase of *A. hydrophila* infection in tilapia as this study proved that red swamp crayfish (*Procambarus clarkii)* can act as a carrier for such virulent bacterial pathogen. In addition, the isolated *A. hydrophila* from crayfish can lead to MAS lesions in *Oreochromis niloticus.*

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