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Histopathological Alterations in Estuarine Catfish *(Arius maculatus*; Thunberg, 1792) Due to *Aeronomas hydrophila* Infection

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Abstract: Bacterial pathogens may cause a serious loss in aquaculture and also health hazards to humans. Infection with *Aeromonas hydrophila* in estuarine catfish *Arius maculatus* induced hemorrhages at the base of fins, erosion of the pectoral fins, ulcers on the skin surface and septicemia after 24 hours of infection. Internal signs of infection includes: fluid in the abdomen, swollen liver, spleen and kidney with clear histopathological alterations in morbid fishes. It was concluded infection studies may clearly stated that the sign of infection and their impact on consumption. Moreover histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants.

Key words: Catfish % Hemorrhages % Aeromonas % Spleen % Gills % Melanomacrophage

INTRODUCTION

Aeromonas hydrophila is regarded as the most important bacteria for causing "aeromonosis" or "motile Aeromonas septicemia" and is the causal agent of haemorrhagic septicaemia [1]. Aeromonads primarily cause disease in cultured warm water fishes: minnows, bait fishes, carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*), striped bass (*Morone saxatilis*), largemouth bass (*Micropterus salmoides*) and tilapia. Also, the pathogen may affect a variety of cool and cold-water species, but is not necessarily restricted to fresh water environments. Rahim *et al.* [2] had isolated *A. hydrophila* from wounds of five species of brackish water fish including species; *Platosus anguillaris, Lates calcarifer*, *Epinephelus; megachir*, *Labeo ruhita* and *Serotherodon nilotica*. Thampuran *et al.* [3] had not only isolated motile aeromonads from raw and processed products of marine fish, but also from marine fishing grounds, as well.

Infectious diseases reduce catfish production by nearly 10% every year. Motile aeromonad septicemia, caused by *Aeromonas hydrophila*, is one of the common diseases accounting for the reduced production. In humans, it has been associated with gastroenteritis and localized wound infection, but the primary concern from a public health standpoint is for individuals who have an immune deficiency [4].

Considering the susceptibility of fish to various potential stressors, it would be advisable to conduct regular health studies on the fish. The present investigation was planed to study the occurrence and symptoms and the histopathological alterations in Aeromonas infectedin Cat fish (*Arius maculatus*) from south east coast of India

MATERIALS AND METHODS

Fish: Twenty catfish (*Arius maculatus*) weighing between 145-180 g were obtained from velar estuary, Parangipettai, South east coast of India. Fishes were acclimatized in fiber tanks with UV treated Sea water. The salinity range was from 22-30 ppt with a temperature between 25°-30°C, fed with commercial fish pellet (4% body weight). The water in the tank was continuously aerated and re-circulated using a biofilter. Daily 15% of the total water volume was replaced by fresh aerated sea water.

Bacterial Culture: Bacterial pathogen *A. hydrophila* subspecies hydrophila 646 MTCC was obtained from IMTECH, Chandigarh, India and the culture was

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maintained as glycerol stocks (40% W/V) at-20°C for routine work.

Infection Studies: To determine LD50 concentration and pathogenicity of the pathogen *A. hydrophila*, the bacterium was cultured in the laboratory in LB broth at 37°C for 24 h and then the cells were separated by centrifugation at 8,000 × g and (104, 105, 106, 107 and 108 cells/ml) injected into the fishes (size 50-60 g) intraperitoneally at room temperature by following the method of Saeed and Plumb [5]. The infected fishes were collected and immobilized. Spleens were removed and fixed in phosphate buffered formalin. Paraffin thin sections at 6 Fm were stained by the standard hematoxylin and eosin [6].

RESULTS

Clinically-infected cat fishes exhibit hemorrhages at the base of fins, erosion of the pectoral fins, ulcers on the skin surface and septicemia after 24 hours of infection. Control gills shown the clear gill filament (GF), secondary lamellae (SL), nucleated erythrocyte (Er) (Fig.1A). Gills of infected fish after 24 hours completely collapsed GF and damaged secondary lamellae (DSL) (Fig.1B). Completely detached filament degraded secondary lamellae (shown as arrow) and clusters of bacterial cells (BC) in filament (Fig. 1C.).

The spleen of cat fish is a reddish-brown, elongated, thick and flattened structure, lying along the intestine and in the proximity of the pancreas, trunk-kidney. The spaces

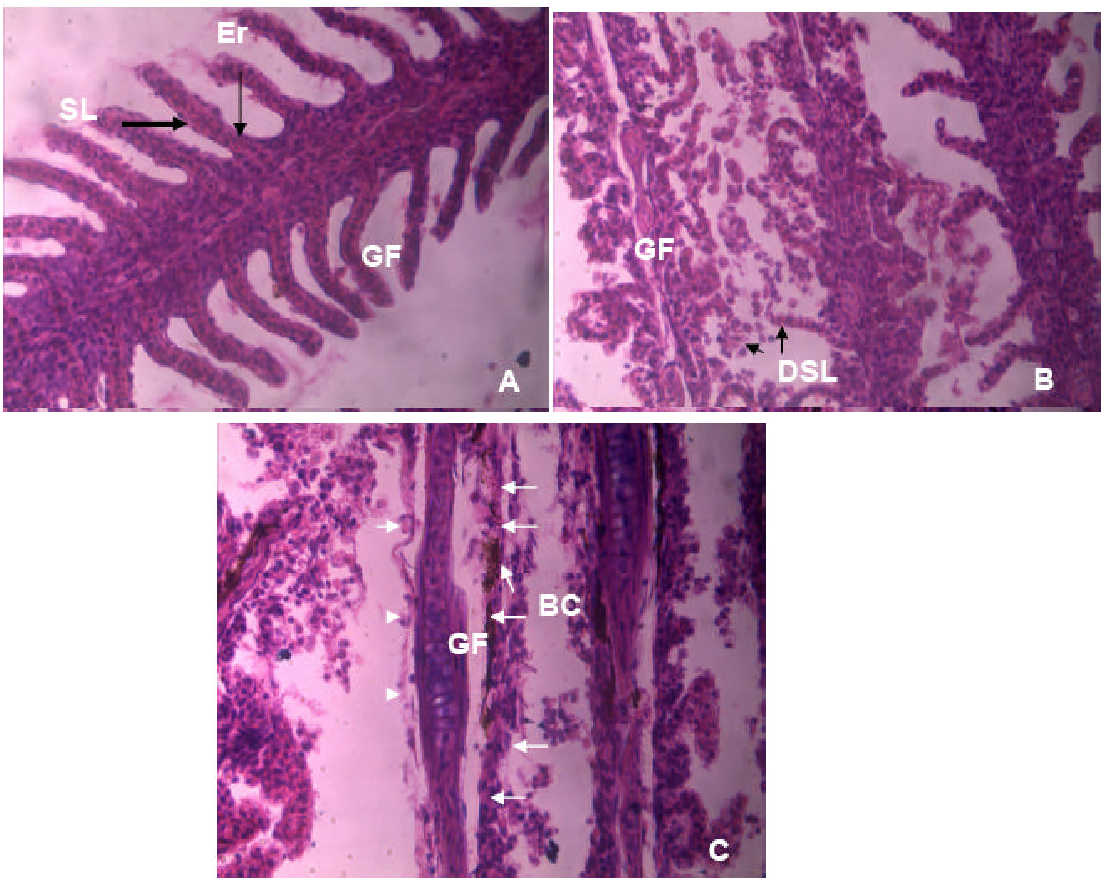


Fig. 1: Light micrograph of gills of estuarine catfish, *Arius maculates*:

A. Control group showing gill filament (GF), secondary lamellae (SL), nucleated erythrocyte (Er). Hematoxylin and Eosin. X 100.

B. Gills of infected fish after 24 hours, completely collapsed GF and damaged secondary lamellae (DSL). Hematoxylin and Eosin. X 100.

C. Completely detached filament, dissociated secondary lamellae (shown as arrow) and clusters of bacterial cells (BC) in filament. Hematoxylin and Eosin. X 400.

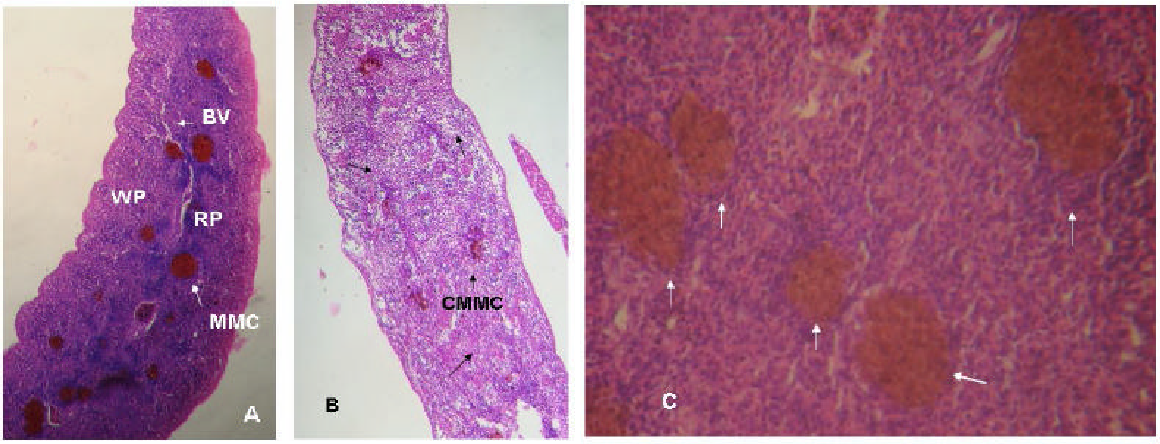


Fig. 2: Light micrographs of spleen of estuarine catfish, *Arius maculatus*:
A. control section shows red pulp (RP), white pulp (WP), blood vessel (BV) consisting of elastic fibers and Malenomacrophage centers (MMC).
B. Bacteria infected spleen shows loosely packed red and white pulp (arrow) and collapsed MMC which shows heavy infection due to *A hydrophila*. Hematoxylin and Eosin. X 100. C. arrows shows malenomacrophage centers i.e, clusters of macrophage cells in control spleen Hematoxylin and Eosin. X 400.

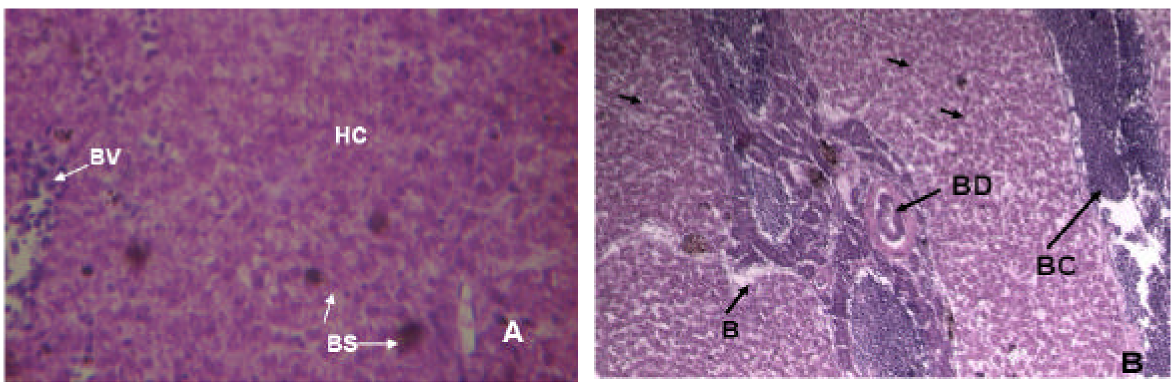
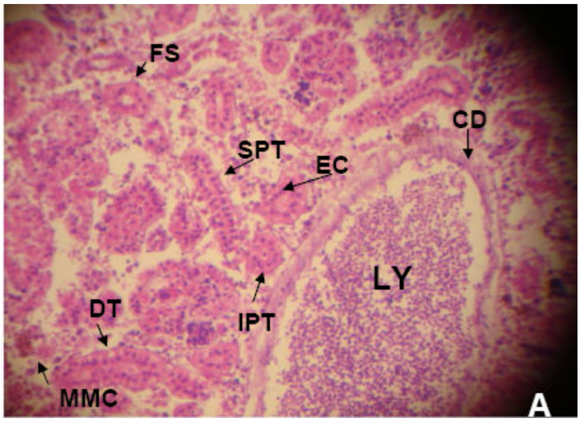


Fig. 3: Light micrographs of Liver of estuarine catfish, *Arius maculatus*:
A: Control group showing normal hepatocytes (HC), Blood vessels (BV) and Blood sinusoids (BS).
B. After infection with *A.hydophila*, cells of hepatocytes are completely damaged (arrowheads) and influx of blood cells (BC) in capillaries. Bile ducts (BD) connected to tissues and macrophages (Kupffer cells). Hametoxlin Eosin, 400X.

within the connective tissue framework are filled with splenic pulp, the distinction of which into red pulp and white pulp is rather difficult in histological preparations. The pulp in general consists of large venous sinuses and their branches, representing the red pulp (RP), white pulp (WP), nodule-like ellipsoids of lymphoid tissue and dark-staining melanomacrophage centres (Fig. 2A). *A. hydrophila* infected spleen shows loosely packed red and white pulp (arrow) (Fig.2B) and collapsed MMC which shows heavy infection (2C).

The liver is a largest of the extramural organs which include hematopoietic tissue and macrophage aggregates. Control group of fish shown normal hepatocytes (HC), Blood vessels (BV) and Blood sinusoids (BS) (Fig.3A). Blood sinusoids are lined with reticulo-endothelial cells which are surrounded by hepatocytes. After infection with *A. hydophila*, cells of hepatocytes are completely damaged (arrowheads) and influx of blood cells (BC) in capillaries (Fig.3B). Blood congestion in sinusoids and hydropic swelling of hepatocytes were observed.



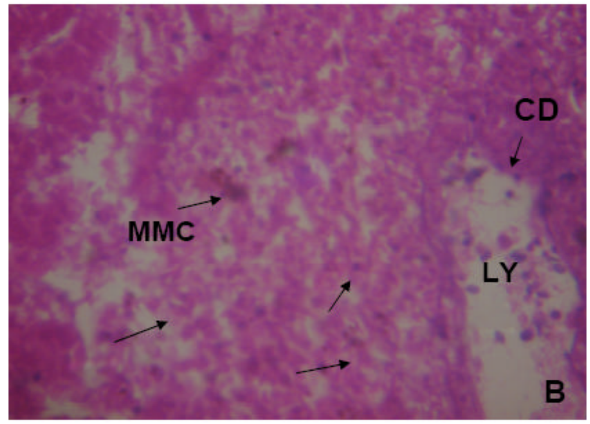


Fig. 4: Light micrographs of Liver of estuarine catfish, *Arius maculatus*:
A: Control group showing normal glomerulus's First proximal tubule (FPT), Second proximal tubule (SPT), Intermediate tubule segment (IPT), Distal tubule (DT), Collecting duct (CD); epithelial cell (EC), Melanomacrophage (MMC), Lymphocytes (LY).
B: Infection induced fish kidney showing entirely destroyed tubules (arrowheads), collecting duct also infected and there is no lymphocytes and macrophage centres (MMC) Hametoxlin Eosin, 400X.

Kidney tubules (Fig. 4A) consists of the first proximal segment (FPT) is covered by tall columnar epithelial cells with basal nuclei and slightly eosinophilic cytoplasm. An apical "brush border" of microvilli protrudes deeply into the lumen. The second proximal segment (SPT) has a still taller columnar epithelium with more centrally located nuclei and a less well developed brush border. The intermediate segment (IPT), long and well developed in the fathead minnow, has a narrow lumen surrounded by cuboidal to short columnar epithelial cells with an inconspicuous brush border. The distal segment (DT) is lined with large, relatively clear columnar epithelial cells. Infection induced fish kidney showing entirely destroyed tubules (arrowheads), collecting duct also affected and there is no lymphocytes and macrophage centers (MMC) (Fig.4B).

DISCUSSION

The south Asian countries are inhabited by over 930 species of which Siluroidei (catfish) comprise about 142 species belonging to 13 families and 46 gencra. Except for the Plotosidae and Ariidae, which have marine representatives, they are largely a group of freshwater fishes like *Mystus* sp (Tripathi 1996).

A. hydrophila has received particular attention because of its association with human diseases. Hazen *et al.* [7] concluded that although *A. hydrophila* was not generally considered to be a marine bacterium, it could be found naturally in marine systems which interface with fresh water and at all salinities except the most extreme. In American channel catfish farms outbreaks of motile aeromonad septicemia due to the potential opportunistic pathogen *A. hydrophila* are experienced, particularly when fish are exposed to both physical and environmental stressors [8].

Bacterial entry may also be facilitated by injuries as catfish do not have protective scales and their sharp fins can easily tear the skin of another fish within the immediate vicinity. According to the present results, *A. hydrophila* infected catfish showed some symptoms like hemorrhages at the base of fins, erosion of the pectoral fins, ulcers on the skin surface and septicemia. Similarly, Yambot and Inglis [9] described an acute mortality among Nile tilapia in which the most apparent clinical signs included opaqueness in one or both eyes, accompanied by exophthalmia and eventual bursting of the orbit. Others have reported adherence of bacteria to intestine [10] and skin [11] followed by invasion of the liver, spleen, muscle, gills and intestine.

Mortality due to typical histopathological lesions, such as necrosis and atrophy of hepatocytes, necrosis of sheathed arteries in the spleen and necrosis of renal tubules and glomeruli in the kidney has occurred. Deivasigamani [6]stated that humoral and cell mediated immunity have the ability to endeavour immune response against sheep red blood corpuscles (SRBC) in cat fish and also the head kidney is the major antibody producing organ. *A. hydrophila* has been recovered as a pathogen from a wide variety of freshwater fish species, including ornamental fish [12]. It is important to run antibiotic sensitivity tests prior to using antibiotics for controlling Aeromonas out-breaks. Cleanliness and good management practice will reduce Aeromonas out-breaks in a fish production unit [13].

It could be concluded that the infection studies clearly stated the sign of infection and their impact on consumption. Such information confirms that histopathological alterations are good biomarkers for field assessment, in particular in tropical areas that are naturally subject to a multiplicity of environmental conditions.

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