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Research Article

Histological changes brought by *Aeromonas hydrophila* on some cells of spotted murrel, *Channa punctatus* (Bloch)

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Abstract

Channa punctatus was exposed to *Aeromonas hydrophila* for 21 days at a concentration of 10^3 and 10^5 CFU dilutions. Exposure to 7th, 14th and 21st days, the fish showed severe histological changes in the gills, intestine and liver tissues. Initially degenerative changes like hypertrophy of epithelial cells and dilution of the marginal channel and abnormal intestinal villi occurred in the intestine, later disorganization of the serosal layer and hyperplasia, haemorrhagic septicemia of the intestinal villi, liver cord disarray, vacuolation of the cytoplasm and necrosis was observed. Rupture of lamellar epithelium leading to haemorrhage of gills, goblet cell damage and mucous secretion of intestinal villi and degeneration of the liver parenchyma were recorded in an increasing order towards days after exposure. Thus, the histological alterations were, observed according to the hours of exposure to *Aeromonas* sp.

Keywords : *Channa punctatus*, *Aeromonas hydrophila*, liver, intestine, gills and tissues

Introduction

Fishes encounter a variety of pathogens, parasites and pollutants in the aquatic environment (Koca *et al.,* 2008). The histopathological changes in several tissues of fish naturally and experimentally infected with different bacterial strains (Magnadottir *et al*., 2002; Woo *et al*., 2006; Martins *et al*., 2008; Hagiwara *et al*., 2009). *A. hydrophila* has been associated with several disease conditions in fish, including tail rot, fin rot, and haemorrahagic septicaemia. Haemorrahagic septicaemia is characterized by the presence of small surface lesions, often leading to sloughing of the scales, haemorrhagic in the gills and anus; ulcers, abscesses, exophthalmia (bulging eyes), and abdominal swelling (dropsy). Internally, there may be the presence of ascetic fluid in the peritoneal cavity, anaemia, and swelling of the kidney and liver (Miyazaki and Kaige, 1985).

Hagiwara *et al.* (2009) observed the Granulomatous inflammation of the heart, caudal peduncle pectoral and olfactory region in *Serieta dumerili* due to *Streptococcus* injection. However, little information is available on ultrastructural pathology, therefore it is essential to study the histopathalogical impact in tissues like liver, intestine and gills in *Channa punctatus*

Materials and methods

Fish stock acclimatization and maintenance

Channa punctatus is an air-breathing fish and generally a freshwater inhabitant, preferring habitats from stagnant muddy pond water to canals, lakes, rivers, etc. For the present experiment, *C. punctatus* specimens were collected during the summer from a local fish farm in and around Mellapalayam, Tirunelveli District, Tamil Nadu, India. The fishes were transported to the Aqua care Center, Department of Zoology, St. Xavier's college and were kept in cement tank (size: 0.6 m × 0.3 m × 0.3 m, 15 fish per cement tank) under controlled laboratory conditions. The

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average length and weight of the specimen was 9.25 ± 2.5cm and 24 ± 3 gm respectively. Fishes were fed with *Tubifex* sp. and larvae of *Culex* sp. during the acclimatization period only. The water was renewed every day to avoid accumulation of unutilized food or metabolic waste products. Different water parameters like temperature (24 - 26°C) and pH (7.2 - 7.4) were regulated.

Bacterial culture collection and isolation of strains

A. hydrophila was received as lyophilized cultures from MTCC and subsequently revived by adding nutrient broth and transferring the dehydrated culture to a fresh nutrient agar medium. Consequently, the streak plate method was followed to get isolated bacterial colonies on a large part of the agar surface.

Experimental inoculation of *A. hydrophila* on *C. punctatus*

The strains of *A.hydrophila* was cultured in nutrient broth and incubated at 37°C for 24h prior to infectivity testing. Bacterial cells were harvested by centrifugation at 5000rpm for 5 minutes and washed in physiological saline. The strains were enumerated by correlating the optical density (OD) values (600 nm) of the growing culture with the corresponding colony forming units (cfu) obtained by the spread plate dilution method. Different concentrations of bacteria ranging from 10^3 and 10^5 cfu dilutions of *A. hydrophila* mixed with water and introduced into different groups of *C. punctatus*, each containing 10 fish. The fish were observed for changes in their behavioral patterns as well as development of haemorrhagic ulcers and tissue necrosis. The viability of the infected fish was checked at regular intervals of 7^{th}, 14^{th} and 21^{st} days during the experiment (2 groups of fish each containing 10 individuals). The remaining 10 fishes were introduced into normal fresh water as a control. Tissues like gills, liver and intestines were fixed in Bouin's fluid for 24h. Then the tissues were dehydrated by the use of graded alcohol series and Xylene was used as a clearing agent. The tissues were impregnated with wax and microtome sections were cut at 5-6µm thickness and stained in Harris haemotoxylin and eosin.

Results

During experimental period noticeable histological changes in the tissue of liver, intestine and gill of treated fish were observed. The infected specimens were compared with control fish. A section of liver, intestine and gills are shown in Fig. 1 to 12.

Liver

In case of control fish, normal structure and systematic arrangement of hepatocytes were observed (Fig.-13) whereas, *A.hydrophila* infected fishes Showed rupture of blood vessel, pyknosis, mild necrosis and vacuolation. Hemorrhage was also found as appearance of vacuoles in the hepatocytes of fish due to release of blood cells. Fig.- 2 shows Focal necrosis and haemorrhage in the liver, the hepatic parenchyma is made up of two cellular plate surrounded by sinusoids. Between two sinusoids the hepatocytes are arranged as cords. The cords extend between central and portal zone. When the liver of *C. punctatus* exposed to *A. hydrophila* infection, cytoplasmic and nuclear generation was very common. The hepatic tissue exhibited focal necrosis and atrophy in severely infected fish (Fig. 3). Cirrhosis of hepatic tissue and necrotized parenchyma causing haemorrhage with other common pathological symptoms in the liver (Fig.- 4).

Intestine

Histological study of intestine is important for establishing the status of structural integrity. It acts as a tool that helps to improve our understanding of the influence of infective pathogen. Fig.- 5 shows the normal architecture of the intestinal villi, whereas Fig. 6 - 8 shows the gross abnormalities of the intestinal villi of

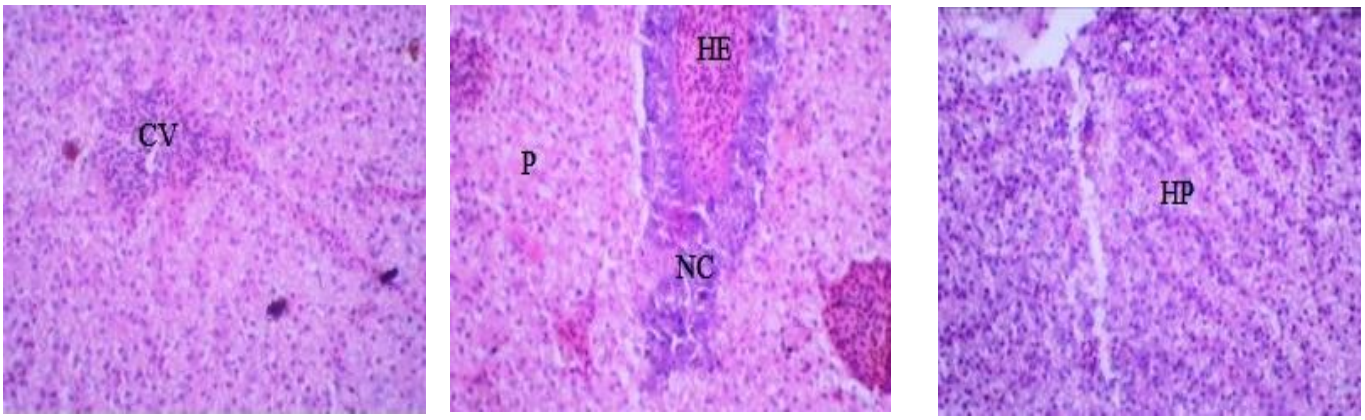

Fig.1. Photograph of the cross section of the liver of control *C. punctatus*, Fig. 2. Cross section of the liver of control *C. punctatus* exposed to *A. hydrophila* for 7 days (40X), Fig. 3. Cross section of the liver of control *C. punctatus* exposed to *A. hydrophila* for 14 days (40X).

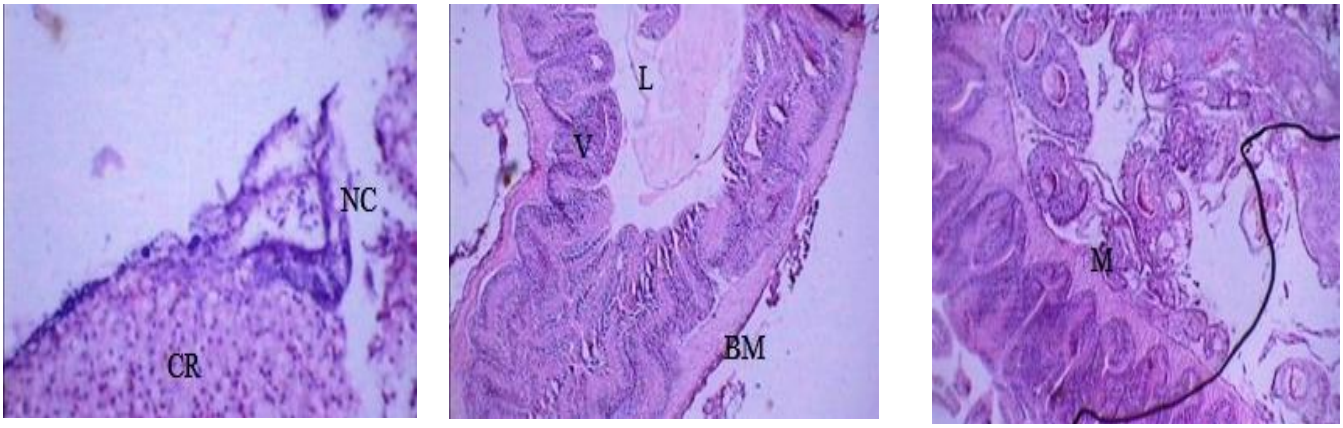

Fig. 4. Cross section of the liver of *C. punctatus* exposed to *A. hydrophila* for 21 days (40X), Fig. 5. Cross section of the intestine of *C. punctatus* (control), Fig. 6. Cross section of the intestine of *C. punctatus* exposed to *A. hydrophila* for 7 days (40X),

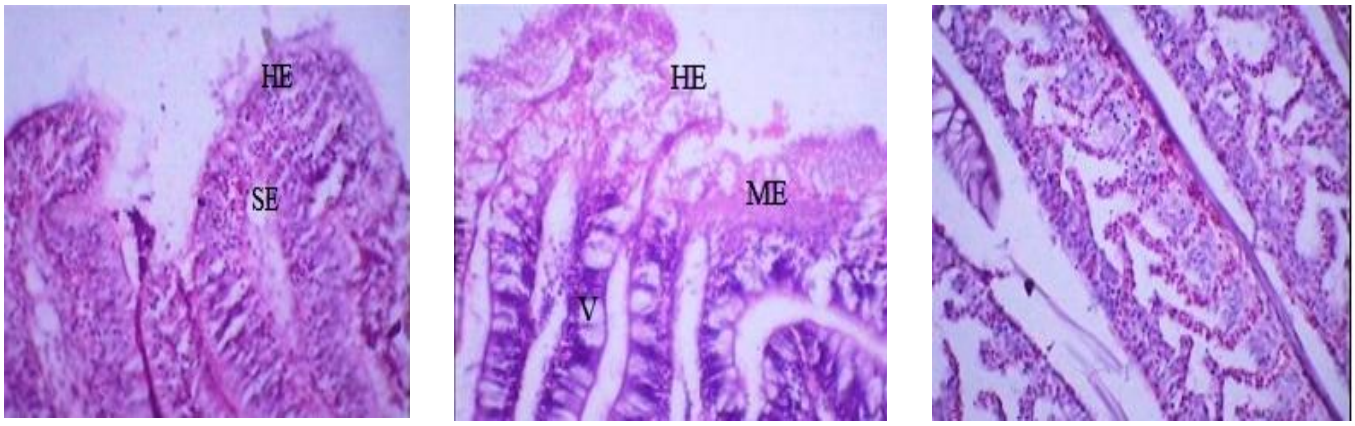

Fig. 7. Cross section of the intestine of *C. punctatus* exposed to *A. hydrophila* for 14 days (40X), Fig. 8. Cross section of the intestine of *C. punctatus* exposed to *A. hydrophila* for 21 days (40X), Fig.9. Cross section of the gill of *C. punctatus* (control)

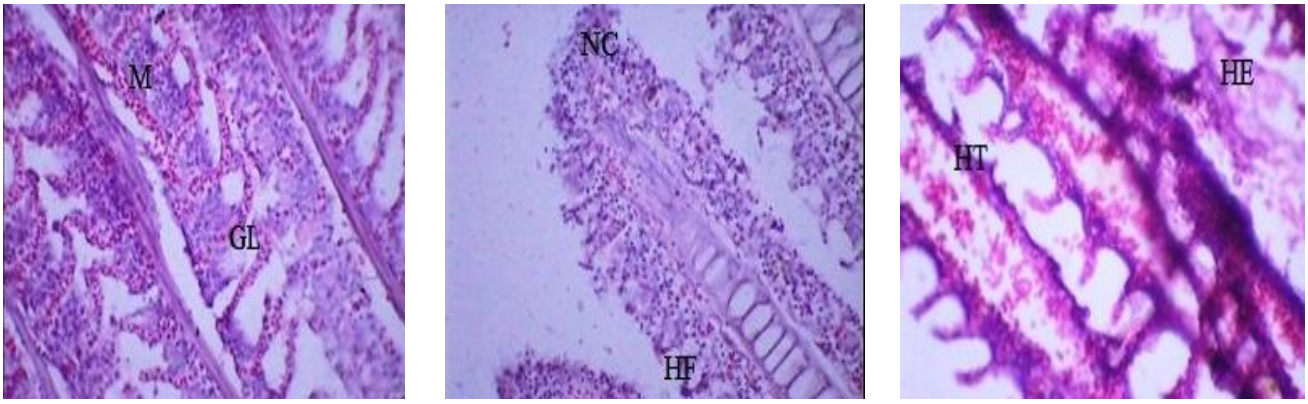

Fig. 10. Cross section of the gill of *C. punctatus* exposed to A. hydrophila for 7 days (40X), Fig. 11. Cross section of the gill of *C. punctatus* exposed to *A. hydrophila* for 14 days (40X), Fig. 12. Cross section of the gill of *C. punctatus* exposed to *A. hydrophila* for 21 days (40X). CR – Cirrhosis; CV – Central Venule; HE – Haemorrhage; HP – Hepatocyte; NC – Necrosis; P – Parenchyma; BM – Basement membrane; L – Lumen; M – Mucous; ME – Mucosal epithelium, SE – Serosa; T – Tubercle; V – Villi, VL – Vacuole; GL – Gill lamella; HT – Hypertrophy; NC - Necrosis

C. punctatus infected with *A. hydrophila*. The gross abnormalities influence basement membrane damage, enlargement of mucous epithelium, goblet cell damage and excessive mucous secretion; disorganization of serosal layer elongation of the columnar cells, bleeding and ulceration of the villi and formation of tubercles in the villi (Fig. 6 and 7). Severe intestinal damage like vacuolation of the mucous epithelium, sloughing of the intestine (Fig.- 6) and necrosis of the mucous epithelium leading to haemorrhagic septicaemia (Fig.- 8) were observed in *C. punctatus* which were continuously exposed to *A. hydrophila* for 21 days.

Gills

The histological alterations found in the gills of *C. punctatus* were shown in the plate from 9 to 12. Histological sections of gill of *C. punctatus* during *A. hydrophila* infection on 7th, 14th and 21st days, exhibited clubbing and fusion of gill filaments, excessive mucous secretion, fusion of secondary gill lamella hypertrophy of epithelial cells and hyperplasia of epithelial cell and the lifting of lamellar epithelium (Fig.-10). On prolonged exposure severe hyperplasia, blood congestion the in primary gill lamella and also rupture of lamellar epithelium leading haemorrhage (Fig.- 11) were observed. In severe infection lamellar aneursyms, haemorrhage and necrosis of gill lamella leading to Bacterial Gill Diseases (BGD) was observed (Fig.- 11 and 12).

Discussion

The pathogenecity of *A. hydrophila* in various freshwater fishes are well documented. *Aeromonas* species is said to produce many products that may be toxic to other cells (Howard and Buckley, 1986). The results of present study led to the contention that *A. hydrophila* may be a primary pathogen of the freshwater fish. Many authors have reported that *A. hydrophila* is one of the most important pathogen of fresh water fishes (Bullock and Malaughin, 1970; Eurell *et al*., 1978). Hagiwara *et al*. (2009) studied the phagocytic system of *A. hydrophila* and the same has been emphasized in the present study that the pathological changes of the disease include exophthalmia, leucopenia, ascites, congestion and enlargement of liver and spleen, congestion in the intestinal mucosal, haemorrhagic liver necrosis and petechiae in the visceral fat.

The gills, which participates in many important function in fish such as respiration,

osmoregulation and excretion, remain in close contact with the external environment and are considered the primary target of contaminants (Fernandes and Mazon, 2003). Countinho and Gokhale (2000) found epithelial lifting in the gills of carps (*Cyprinus carpio*), tilapia *(Oreochromis mossambicus)* exposed to the effluents of a wastewater treatment plant.

Aeromonas caused histological alterations similar to the result caused due to metal exposure like lead in gills and kidney (Martiney *et al*., 2004) An array of protection systems exist within it to limit the risk of damage to the intestine mucosa critical in digestion, absorption and metabolic processes and act as a barrier to pathogenic infection preventing both viable and non-viable bacteria from migrating from the intestinal lumen through the epithelial mucosa (Gernhofer *et al.,* 2001). Mucin and glycoproteins associated with intestinal brush border serve as an important barrier protecting the absorptive surface from bacterial colonization (Gernhofer *et al*., 2001). In the present histopathological study the role of mucosa is clearly visualized. Therefore, the interaction between the intestinal micro flora gut morphology, the immune system and nutrient uptake will have a major influence on the animal health and performance.

The experimental infection by *A. hydrophila* in the present study has resulted in damage to the hepatocytes, cytoplasmic vacuolation, hypertrophy and necrosis. Destructions of tissues in the pronephric kidney of *C. punctatus* at short time exposure to *Aeromonas* was also observed by Ghosh and Homechaudhuri (2012), where changes occurred in the nucleus and cytoplasm. Diffused necrosis in the kidney and histopathological alterations in the liver was observed in *C. punctatus* due to the impact of *A. hydrophila* (El-Barbbary, 2010). Depending upon the hours of exposure *A. hydrophila* induced histological alterations in liver gills and intestinal cells of *C. punctatus.*

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