Histopathology of Aeromonas caviae Infection in Challenged Nile Tilapia Oreochromis niloticus (Linnaeus, 1758)

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Abstract Aeromonas caviae is one of the predominant motile aeromonads in Nile tilapia, Oreochromis niloticus. In this study, the histopathological alterations in the kidney, liver and pancreas of O. niloticus juveniles experimentally challenged with u-haemolytic A. caviae is described. The Nile tilapia experienced 60% mortalities at a challenge dose of 6x10⁶ cells/fish. Post-challenge, well-defined histopathological changes were observed with nephritis and the loss of structural integrity of the kidney tissues. The liver was dispersed, necrotized and had fatty changes in the hepatic parenchyma. Inflammation of the pancreas, as well as pancreatic acinar cells and disintegration of intrahepatic exocrine pancreatic tissues, were also noted. The results, thus, demonstrated that A. caviae can cause severe damages in the kidney, liver and pancreas of O. niloticus, similar to those of other known fish bacterial pathogens.

Keywords Aeromonas caviae; Oreochromis niloticus; Histopathology; Nephropathy; Inflammation

Background

Disease is one of the major problems that affect the tilapia production and livelihood of the farmers. The bacteria that cause diseases and mortalities in tilapia are Flavobacterium columnare, Edwardsiella tarda, Aeromonas spp., Vibrio spp., Francisella spp., Streptococcus agalactiae, etc (Zamri-Saad et al., 2014; Huicab-Pech et al., 2016).

The genus Aeromonas includes ubiquitous bacteria found in aquatic habitats and at least 31 species have been described (Chen et al., 2016). Aeromonas spp. are considered to be opportunistic pathogens and capable of producing disease only in weakened populations of fish or as secondary invaders in fish suffering from other diseases (Harikrishnan and Balasundaram, 2005; Austin and Austin, 2012). Aeromonas spp. are divided into two principal subgroups: the non-motile and psychrophilic species (A. salmonicida) and the motile and mesophilic species namely A. hydrophila, A. sobria, A. veronii, A. caviae and others (Austin and Austin, 2012). Several species of the genus Aeromonas frequently cause problems in both feral and cultured fish and are responsible for heavy economic losses due to high mortality (El-Sayed, 2006; Martins et al., 2008; Austin and Austin, 2012).

The Nile tilapia O. niloticus is reportedly sensitive to streptococcosis and also motile Aeromonas septicemia (MAS). Diseases associated with streptococcosis and MAS are, however, most severe among fish that are cultured under intensive conditions (El-Sayed, 2006; Martins et al., 2008; Zamri-Saad et al., 2014). In a study by Martins et al. (2008), A. caviae was associated with mortalities of Nile tilapia held in cages, wherein they isolated large numbers A. caviae in the liver and kidney. Saleh et al. (2017) reported A. caviae as one of the predominant species of motile aeromonads in Nile tilapia. As the Nile tilapia culture is fast picking up, we started investigating the diseases of cultured tilapia in West Bengal, India. In our earlier studies, streptococcal (Adikesavalu et al., 2017) and A. hydrophila (Julinta et al., 2017) infections in Nile tilapia were reported. In this study, we report the histopathological alterations in the kidney, liver and pancreas of O. niloticus juveniles caused by experimental A. caviae infection.

1 Materials and Methods

1.1 Bacterial challenge in Oreochromis niloticus juveniles

Healthy Oreochromis niloticus (14.50 ± 0.50 g; 11.20 ± 0.57 cm) were brought from Naihati, North 24 Parganas
An α-haemolytic bacterium, Aeromonas caviae-T1K2 used in this study was isolated from diseased O. niloticus with MAS and preserved as glycerol stock at -20°C in the Department of Aquatic Animal Health, West Bengal University of Animal and Fishery Sciences, Kolkata, India. The glycerol stock was revived in tryptic soya broth and the cell suspension of A. caviae was prepared as described in Adikesavalu et al. (2015) and used immediately. Our preliminary studies with A. caviae-T1K2 determined the LD₅₀ value in Nile tilapia juveniles as 6.76×10⁸ cells/fish (data not shown). Aliquots (0.1 mL) of the undiluted A. caviae cell suspension (6×10⁹ cells/mL) was injected intramuscularly, i.e., on the dorsal side of the body at a 45° angle at the base of dorsal fin, in such a way to get 6×10⁸ cells/fish. The control fish received sterile saline, 0.1 mL each. The challenged fish were maintained in their respective tanks for the observations on mortality, external signs of infections and behavioural changes.

1.2 Histopathology
The organs such as kidney and liver of the freshly dead O. niloticus on day 4 post-injection were fixed in Bouin’s solution for 24 h. The fixed organs were processed, embedded in paraffin wax and thin (5 µm) sections prepared. The sections were then stained with haematoxylin and eosin for the detection of histopathological changes as per Roberts (2012).

2 Results
Aeromonas caviae challenged O. niloticus exhibited sluggishness and abnormal behaviour like wandering around the corners, resting at the bottom and vertical swimming. On 4-day post-injection (dpi), 60±6% of the challenged tilapia died. The histopathological changes noted in the kidney tissues of A. caviae challenged O. niloticus are depicted in Figures 1a-c, and those of liver and pancreas in Figure 2a-c and Figure 3a-c, respectively. The kidney tissues had glomerulopathy with dilated Bowman’s space, nephropathy with the loss of tubular epithelial cells, obliterated as well as inflamed nephritic tubules, melanomacrophage aggregate, necrosis, widen lumen and thickening of lumen lining (Figures 1a-c). The liver demonstrated melanomacrophage aggregate, infiltration of haemocytes, loss of normal architecture of the hepatic tissue and fatty changes in the hepatic parenchyma; while the pancreas illustrated degradation and/or inflammation of the pancreas and pancreatic acinar cells and, vacuolation in the pancreatic tissue (Figure 2a-c; Figure 3a-c).

3 Discussion
Aeromonas caviae strain used in this study was isolated from the kidney of septicemic O. niloticus along with other motile aeromonads such as A. hydrophila, A. veronii, A. schubertii and A. bestiarum (data not shown). The challenged tilapia showed haemorrhages at the site of injection and experienced 60% mortalities. The main visceral organs, viz., kidney, liver and pancreas were affected in the injected fish, thus revealing the fact that A. caviae was able to infect many visceral organs similar to Aeromonas caviae-like bacterium (Thomas et al., 2013) and A. hydrophila (Azad et al., 2001). Systemic infections are generally characterized by diffused necrosis in several internal organs, primarily the liver and kidney are the target organs of an acute septicemia. These organs when attacked by bacterial toxins cause acute haemorrhage and necrosis of vital organs and lose their structural integrity (Huizinga et al., 1979). In control fish, the normal structure and systematic arrangement of kidney tissues with well-defined glomerulus were observed. While, in challenged tilapia, the structural integrity of the kidney tissues were lost and well-defined histopathological changes observed on 4 dpi, which indicated disease
progression with extensive changes in the tissues of this vital organ. The inflammation of nephritic tubules of challenged tilapia exemplified nephritis. The glomerulopathy and dilation of Bowman’s space are the indications of a defective glomerular filtration of blood, which, in turn, hamper the removal of excess wastes and fluids from the kidney. This clearly justified the ability of A. caviae in causing systemic infection as it contains many putative virulence genes, including those encoding a type 2 secretion system, an RTX toxin, and polar flagella (Sudheesh et al., 2012). The findings of this study are reasonably similar to those observed by Julinta et al. (2017) in O. niloticus intramuscularly challenged with A. hydrophila, a strain isolated along with A. caviae strain used here. On the other hand, in A. hydrophila challenged O. mossambicus, Azad et al. (2001) noted aggregation of melanmacrophage centers (MMC) in the pronephros, necrosis of the cells in the renal interstitium, tubular necrosis and glomerular degeneration, oedematous degeneration of the tubules, depletion of cells in the tubular interstitium occlusion of the ophisthonephric collecting duct with MMC.

Figure 1 Photomicrography of the kidney tissues of Oreochromis niloticus intramuscularly infected with Aeromonas caviae-T1K2 showing [a] normal architecture X400 H&E staining; [b] melanomacrophage aggregate (MA), glomerulopathy (G) with dilated Bowman’s space (BS), nephropathy with the loss of tubular epithelial cells (LE), inflamed (I) and obliterated nephritic tubules (O), widen lumen (W), necrosis (N) X200 H&E staining and [c] glomerulopathy (G), nephropathy with the loss of tubular epithelial cells (LE), widen lumen (W), necrosis (N), inflamed nephritic tubules (I) and thickening of lumen lining (TL) X200 H&E staining

Figure 2 Photomicrography of the liver tissues of Oreochromis niloticus intramuscularly infected with Aeromonas caviae-T1K2 showing [a] normal architecture X200 H&E staining; [b] dispersed (D) and necrotized (N) tissue, and fatty changes in the hepatic parenchyma (F) X100 H&E staining and [c] melanomacrophage aggregate (MA), infiltration of haemocytes (IR) and loss of normal architecture of the hepatic tissue X200 H&E staining

The liver of the control fish showed a normal structure and systematic arrangement of hepatocytes. The pancreatic tissue inside the liver is not common in all kinds of fish, but if it is, this organ is called the hepatopancreas (El-Bakary and El-Gammal, 2010). The observations of this study revealed the presence of pancreatic tissue inside the liver of O. niloticus. In the liver and pancreas of challenged tilapia, dispersed and necrotized tissue, infiltration of haemocytes, loss of normal architecture of the hepatic tissue, fatty changes in the hepatic parenchyma, inflammation of pancreas as well as pancreatic acinar cells, and disintegration of intrahepatic exocrine pancreatic tissues were commonly observed, which corroborate the observations of several earlier studies conducted on different fish species due to Aeromonas infection (Azad et al., 2001; Ghosh and Homechaudhuri, 2012; Al-Yahya et al., 2018). Azad et al. (2001) documented vacuolation, congestion of hepatic sinuses with blood cells and internal haemorrhages, pyknotic necrosis of hepatocytes in the liver of A. hydrophila challenged O. mossambicus; while in the pancreas, they observed acinar cell degradation in 3-5 dpi and mild necrosis in the pancreatic acini. On the other hand, Al-Yahya et al. (2018) noted massive haemocyte aggregation, pyknotic nuclei in the hepatopancreas and perivascular cuffing of hepatopancreatic haemolymph vessels in A. hydrophila infected blue
tilapia, *O. aureus*. These changes may lead to a disorder of lipid metabolism in the liver tissues, i.e., lipidosis, possibly associated with toxins and extracellular products such as hemolysin, protease, elastase produced by aeromonads (Yardimci and Aydin, 2011). In contrast, the study by Islam et al. (2008) revealed the development of internal tissue abscess characterized by focal necrosis and haemorrhage. According to them, the distribution of bacterial cells all over the hepatic tissue caused massive diffused necrosis represented by vacuolation and atrophy in the liver of fish challenged with *Aeromonas*. The infiltration of haemocytes in the hepatic tissue is a measure of cellular response, which indicated the ability of Nile tilapia to respond to the *A. caviae* infection. The results of the present study, thus, demonstrated that *A. caviae* can cause serious pathology in the kidney, liver and pancreas of *O. niloticus*, similar to those of other known fish bacterial pathogens such as *A. hydrophila* (Azad et al., 2001; Julinta et al., 2017; Al-Yahya et al., 2018) or *S. agalactiae* (Adikesavalu et al., 2017).

Figure 3 Photomicrography of the pancreas tissues of *Oreochromis niloticus* intramuscularly infected with *Aeromonas caviae*-T<sub>1</sub>K<sub>2</sub> showing [a] normal architecture X400 H&E staining; [b] inflamed pancreas (I), vacuolation in the pancreatic tissue (V), fatty changes in the hepatic parenchyma (F) X400 H&E staining and [c] vacuolation (V), degradation (D), inflammation of pancreatic acinar cells (I) and fatty changes in the hepatic parenchyma (F) X400 H&E staining

Since *A. caviae* caused mortalities only at a challenge dose of 6×10<sup>8</sup> cells/fish, it is highly apparent that risk factors such as temperature fluctuations, poor water quality, accumulation of organics, crowding, etc, together with other virulent motile aeromonads may affect the physiological functioning of tilapia and increase their susceptibility to the pathogenic agents. Therefore, the search for timely corrective measures should first address the identification of risk factor(s) that predispose *Aeromonas* outbreaks in Nile tilapia and mitigating the same responsibly.

**Authors’ contributions**

TJA contributed to conception, analysis and interpretation of results, and write-up of the manuscript. AR and JS contributed to sample collection, analysis, histology as well as acquisition of data. All the authors read and approved the final manuscript.

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