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Immuno-pathological changes in Indian catfish *Clarias magur* (Hamilton, 1822) upon experimental challenge with *Aeromonas hydrophila*

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ABSTRACT

Motile *Aeromonas* septicaemia (MAS), caused by *Aeromonas hydrophila*, is one of the most significant problem among the bacterial diseases responsible for causing mortality in catfishes. The objective this study was to understand the modulation of innate immune parameters and histopathological alteration in Indian catfish *Clarias magur* (Hamilton, 1822), following intraperitoneal injection with *A. hydrophila*. At 3 to 8 h post-infection (hpi), respiratory burst activity, myeloperoxidase, protease, total anti-protease and α2 macroglobulin increased significantly ($p<0.05$) indicating immune response of host against pathogen invasion at an early stage of infection. The total protein, albumin, albumin/globulin ratio and bactericidal activity were significantly decreased indicating the response of pathogen to suppress host immune response to establish infection. Serum bactericidal activity, bacterial agglutination titre and lysozyme activity increased significantly at 24 hpi. Histopathological examination of infected fish revealed the accumulation of melanomacrophage centres, congestion, hepatocyte degeneration and vacuolated hepatocytes of liver; hyaline droplet accumulation, lymphocytes infiltration and diffuse necrosis of renal tubule in kidney; unilateral fusion of secondary gill lamellae, leukocytic infiltration as well as dilated central venous sinus in gills. Knowledge regarding the immune response in magur could be useful for developing strategies for improving disease resistance against *A. hydrophila* infection.

Keywords: *Aeromonas hydrophila,* Histopathological alterations, Indian catfish, Innate immune parameters, Intraperitoneal challenge

Introduction

Gram-negative motile pleomorphic bacillus *Aeromonas hydrophila,* is the causative agent of motile *Aeromonas* septicaemia (MAS), a bacterial disease affecting the feral fish and cultivated freshwater aquaculture fish species including catfish, carp, salmon, trout (Harikrishnan *et al.,* 2003). Different life stages of magur are affected by *A. hydrophila* infection and the mortality may go up to 70-80% during rearing stages, whereas 50% mortality has been recorded in grow-out ponds (Sinha *et al.,* 2014; Sharma *et al.,* 2018). The innate immunity that is an inherent and fundamental defence mechanism of fish offers immediate protection against a wide array of pathogens (Magnadottir, 2006). Acute phase response protein such as lysozyme, ceruloplasmin, myeloperoxidase, protease, anti-protease and complement component are key players of innate immunity in response to pathogen infection and some are linked with resistance to bacterial diseases (Sahoo *et al.,* 2011). On the other hand, pathogens continuously dispose of virulence factors to subvert host defences for their benefit and thereby helping to establish infection (Banfield and Kamoun, 2013).

A study of the literature indicates that there is paucity of information regarding modulation of the host innate immune response and pathological changes at the tissue level in magur in response to *A. hydrophila* infection. The aim of the present work was to study the sequential changes in innate immune parameters and histopathological alterations in *C. magur* following an intra-peritoneal challenge with *A. hydrophila.* The information gathered on sequential changes in innate immune parameters in response to *A. hydrophila* can divulge mechanisms of host-pathogen interaction which could be useful for developing future disease management or control strategies against this pathogen.

Materials and methods

Experimental fish and maintenance

Apparently healthy juveniles of *C. magur* (n=120; 22.31±1.34 cm; 50±4.54 g) were collected from nearby farms in Lucknow and stocked in 2000 l capacity fibre reinforced plastic (FRP) tank with sandy-clay bed and hideouts. Fish were fed with commercially available magur feed (Growel Feed Pvt. Ltd, India) @ 2%

of their body weight twice a day. Fishes were acclimatised for a period of 1 month before carrying out the experiment.

Experimental design

One hundred twenty numbers (n=120) of *C. magur* were randomly distributed in an equal number from acclimatisation tank into three experimental tanks (control; *A. hydrophila* challenge and mock-challenge tanks) of 1000 l capacity FRP tank. Before initiation of the experiment, three fish were sampled randomly for bacterial and parasitological examination to ensure that fish were free of bacterial and parasitic infection. During the experimental period, the mean values of temperature, dissolved oxygen and pH recorded for water in the experimental tanks were 35±2°C, 6.8±0.78 mg l^{-1}, 8.2±0.45, respectively as measured by a multiparameter water quality meter (Hanna Instruments, Romania), whereas, nitrite and ammonia were estimated to be 0.013±0.007 mg l^{-1} and 0.106±0.02 mg l^{-1}, respectively (APHA. 1998).

Our previously isolated and characterised *A. hydrophila* strain (strain ID 9C) was used for this experiment. Experimental group (*A. hydrophila* challenge, n=40) as well as the mock-challenged group (n = 40) of fishes were injected with lethal dose (LD_{50}) of *A. hydrophila* at 1×10^5 cfu per fish in 100 µl of sterile phosphate buffered saline (PBS) by intra-peritoneal route and control group (n=40) was injected with same volume of sterile PBS. Three fishes were randomly sampled from experimental and control tanks at different time points *i.e.* 3, 8, 24, 72 and 144 h post-infection (hpi) challenge. Re-isolation of bacteria from kidney of moribund magur in experimental group was carried out to confirm the cause of mortality.

Blood, serum and tissue sample collection

Fish were anesthetised with clove oil (200 ppm) and bled from the caudal vein using a 2 ml syringe. An aliquot of blood was drawn using heparin (50 IU ml^{-1}) rinsed syringe, without coagulation. For serum separation, non-heparinised blood was collected into a sterile 5 ml glass vial and allowed to clot at room temperature for 1 h for release of the serum. Separated serum was stored at -80ºC until further use. For histopathological examination, tissue specimens of kidney, liver and gill were excised and fixed in 10% neutral buffered formalin (NBF). The animal care and experimental challenge was approved by the Institutional Animal Ethics Committee (NBFGR/IAEC/2019/007) of ICAR-NBFGR, Lucknow.

Innate immune parameters

The respiratory burst activity was measured by reduction of nitroblue tetrazolium dye following the protocol described by Anderson and Siwicki (1995). The serum myeloperoxidase content was measured according to Quade and Roth (1997). Total serum protein content was estimated as per the method described by Bradford (1976). Serum albumin content was determined following bromocresol green (BCG) dye binding method described by Doumas *et al.* (1971), using albumin kit (Siemen Ltd. India). Globulin value was calculated by subtracting albumin value from total protein. Protease activity in serum was quantified using Azocasein hydrolysis assay according to method followed by Guardiola *et al.* (2014). Total anti-protease activity in serum was quantified using trypsin inhibition assay according to method followed by Guardiola *et al.* (2014). The alpha-2 macroglobulin activity of serum was estimated as described by Zuo and Woo (1997). *In-vitro* bactericidal activity of serum was performed following the procedure described by Kajita *et al*. (1990). Lysozyme activity in serum was determined according to Ellis (1990). Bacterial agglutination titre assay was performed following Sahoo and Mukherjee (2002).

Histopathology

Fixed tissue specimens were kept under constant water flow overnight followed by dehydration in a series of ascending alcohol concentration (70, 80, 90 and 100%), embedded in paraffin wax and sectioned at 5 µm in rotary microtome. Tissue sections were stained with haematoxylin-eosin (H&E) and examined under a compound microscope (Olympus BX53, Tokyo, Japan).

Statistical analysis

Data were compared by one way ANOVA followed by Tukey's honest significance at 95% level using statistical package SPSS version 16 and expressed as mean±standard error of the mean (SEM). A value of $p \leq 0.05$ considered as statistically significant.

Results and discussion

Clinical signs and mortality

No clinical signs and mortality were observed in fish of the control group throughout the experimental period. In the experimentally infected group, no clinical signs were observed up to 3 hpi, whereas, by 8 hpi, fish started exhibiting lethargy, copious mucus secretion and erratic swimming. By 24 hpi, the clinical sign increased in severity in infected fish which included hyperemia, petechiation and mild ascites, blood discharge from vent and haemorrhages over various body parts *i.e.* pectoral and pelvic fins, barbels, dorsal and ventral flank as well as dorsal and caudal fin rot (Fig. 1a and b). *Aeromonas* spp. can attack the fin, tegument and abdominal cavity and may be able to break the blood vessels, resulting in ulcerative lesions with a hemorrhagic appearance throughout the

Fig. 1. a and b. Gross pathology of *A. hydrophila* infected *C. magur* showing blood discharge from vent, dorsal and caudal fin rot and haemorrhages over various body parts *i.e.* pectoral and pelvic fins, barbels, dorsal and ventral flank

tegument (Doan, 2013). In the mock challenge tank, fish started dying from 12 hpi and by 72 hpi, a cumulative mortality of 50% (20 fish) was observed. Out of the 40 fish, twelve fish (30%) died by 12-24 hpi, whereas, six fish (15%) died during 24-48 hpi and two more fish (5%) died during 48-72 hpi. A cumulative mortality of 50% was observed by 72 hpi (Fig. 2). The severity of lesions was found to decrease from 72 hpi and no mortality was recorded after 72 hpi. The clinical signs and gross lesions observed in *A. hydrophila* infected magur are in conformity with previous reports of *A. hydrophila* infection in estuarine catfish, *Arius maculates* (Alagappan *et al.,* 2009), giant fish of Amazon, *Arapiama gigas* (Dias *et al.,* 2016) and channel catfish, *Ictalurus punctatus* (Baumgartner *et al.,* 2017).

Innate immune parameters

Innate immune factors are a vital indicator of the immune status of fish and considered a crucial factor for disease resistance. Respiratory burst and myeloperoxidase (MPO) activities are the major functional indicators of phagocytosis, a potent antimicrobial defence mechanism. The infected group of magur showed significantly higher ($p<0.05$) respiratory burst activity (Fig. 3a) and myeloperoxidase activity (Fig. 3b) at 3, 8, 24 and 72 hpi, compared to control as a sign of host immune protection against *A. hydrophla*. Similar results have been observed following infection with *A. hydrophila* in pacu (Biller-Takahashi *et al.,* 2013), olive barb (Das *et al.,* 2011), estuarine catfish (Jothi *et al.,* 2012), zebra fish (Rodriguez *et al.,* 2008) and in snakehead murrel (Priyadarshini *et al.,* 2017). In spite of significant reactive oxygen species (ROS) production and myeloperoxidase activity at early hour of infection *i.e.* 3 to 24 hpi, 30% mortality of magur occurred by 24 hpi. These findings suggest that, in spite of higher ROS production exhibited by host to overcome *A. hydrophila*, the pathogen is able to evade phagocytosis by detoxification of ROS and hypohalites and cause pathogenesis.

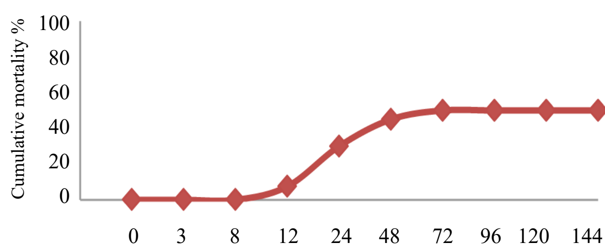

Fig. 2. Mortality pattern of *C. magur* challenged with *A. hydrophila,* during experimental period

The significant decrease in total serum protein (Fig. 3c) and albumin (Fig. 3d) at 3 and 8 hpi imply host immunity suppression at an early period of infection which may be caused by virulence factors secreted by *A. hydrophila*. Increase in serum protein, albumin and globulin levels is thought to be associated with a stronger innate immune response in fish (Wiegertjes *et al.,* 1996). Total protein and globulin (Fig. 3e) content increased significantly at 24, 72 and 144 hpi implying stronger immune response after 24 hpi. A significant decrease in Albumin/Globulin ratio was (Fig. 3f) observed at 24, 72 and 144 hpi. A lower A:G ratio indicates a superior immune status of the fish. Our result is supported by findings of Charlie-Silva *et al.* (2019) who have also reported significant decrease in total protein and albumin at 24 hpi and Pal *et al.* (2016) who reported significant increase in total serum protein, albumin and globulin values in rohu at 3 and 5 days post-infection (dpi) following *A. hydrophila* infection.

Host protease contributes to the natural resistance of fish to infection by directly damaging the pathogen through recognising and cleaving the signature proteins and indirectly facilitating several other immune mechanisms such as complement activation, immunoglobulins or antimicrobial peptides production (Aranishi *et al.,* 1998). In addition, host also secretes several anti-proteases such as α2 antiprotease, α2 antiplasmin and α2- macroglobulin (α2 M) to counter proteases secreted by *A. hydrophila.* Serum protease content was observed to be significantly increased at 3 and 8 hpi compared to the control magur group (Fig. 3g). Significant rise in serum total anti-protease (Fig. 3h) and α2-macroglobulin (Fig. 3i) content was observed at 3, 8 and 24 hpi reaching highest level at 24 hpi. Significant rise in serum protease, total anti-protease and α2-macroglobulin indicates their role in immune magnification and protection from pathogen at early hour of infection. Our result is similar to findings of

Charlie-Silva *et al*. (2019) who reported increase of acute phase proteins *i.e.* α2 macroglobulin in tilapia following *A. hydrophila* infection at 6 to 24 hpi.

A significant increase in serum bactericidal activity (Fig. 3j) was observed at 24, 72, 144 hpi. Bactericidal activity contributed by opsonins, complement cascade and antimicrobial peptides form part of host innate immunity. However, *A. hydrophila* strains resistant to complement-mediated killing (Chena *et al.,* 2019) is directly responsible for the decreased bactericidal activity and increased serum resistance. The *A. hydrophila* 9C strain used in this study has been evaluated to possess serum

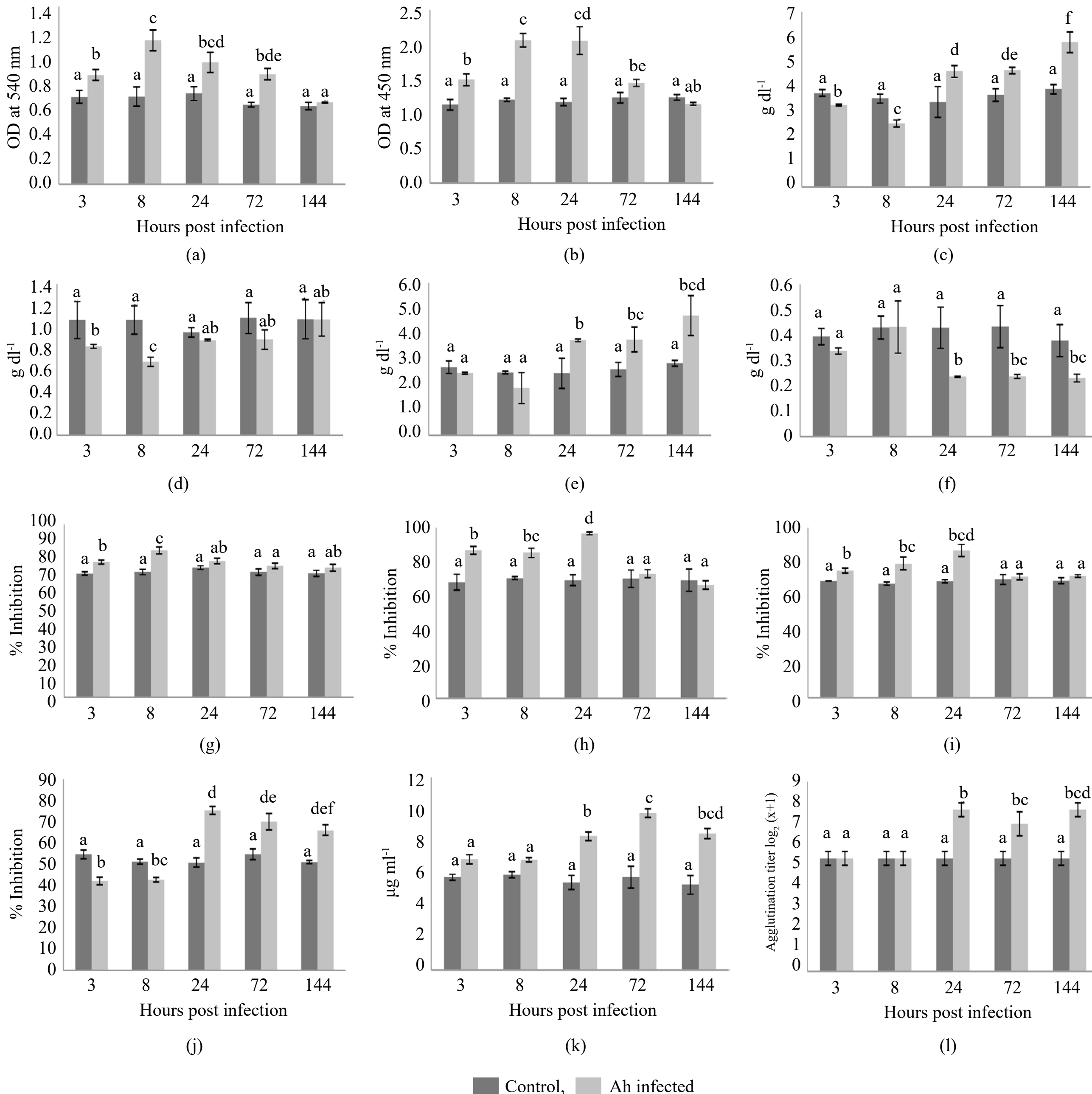

Fig. 3. Effect of *A. hydrophila* infection on various non-specific immune parameters in *C. magur* at different hours post infection; (a) Respiratory burst activity, (b) Myeloperoxidase activity, (c) Total serum proteins, (d) Serum albumin level, (e) Serum albumin level, (f) Albumin/globulin ratio, (g) Protease activity, (h) Total antiproteases, (i) α-2 macroglobulin, (j) Bactericidal activity, (k) Lysozyme activity and (l) Bacterial agglutination titre. Data are presented as mean±S.E. Significant differences (p≤0.05) are indicated by different letters (a, b, c and d) over the bars

resistance properties (Muduli *et al.,* 2020) which might be a reason behind the significant decrease in bactericidal activity of magur at early hour of infection *i.e*. 3 to 8 hpi. However, bactericidal activity was significantly increased at 24, 72 and 144 hpi, which may have contributed to the recovery of the host from bacterial infection. Pal *et al.* (2016) also reported significant increase in bactericidal activity at 3 and 5 dpi following *A. hydrophila* infection.

Lysozyme, a primary defence factor of non-specific humoral immunity, is responsible for bacteriolysis against a wide range of pathogens. Significant increase in lysozyme activity (Fig. 3k) at 24, 72, 144 hpi might have played an important role in containing *A. hydrophila* infection after 24 hpi. Our finding is supported by Priyadarshini *et al.* (2017) who reported significant ($p<0{\cdot}05$) increase in serum lysozyme activity in striped snakehead murrel, *Channa striata* on 5 and 10 dpi and Xia *et al*. (2017) who found significant increase in lysozyme activity at 4 hpi and 1, 3, 5, 14 and 21 dpi in blunt snout bream, *Megalobrama amblycephala* following *A. hydrophila* challenge.

Serum agglutinins and precipitins are lectins like C-type lectins and pentraxins. *Aeromonas* strains have a complex cell surface composed of a mosaic of molecular structures which enable bacteria to bind to a range of biomolecules by lectin like interactions between adhesins and specific glycoconjugates (Ascencio *et al.,* 1990). The serum agglutination titre for inactivated *A. hydrophila* 9C strain in control group of magur was found to be 32 or 64. The bacterial agglutination titre of serum increased significantly to 256 in infected group of magur at 24, 72 and 144 hpi (Fig. 3l). Increased expression of mannose binding lectin has been reported in response to *A. hydrophila* infection (Jiang *et al.,* 2018). No significant differences were observed in any of the immune parameter of the control group during the experimental period of 6 days.

Histopathology

Few studies have been carried out to demarcate histopathological changes following *A. hydrophila* infection in catfish (Alagappan *et al.,* 2009; Abdelhamed *et al.,* 2017). To the best of our knowledge, this study is the first ever report on histopathological changes in magur following *A. hydrophila* infection. In this study, degenerative histopathological changes were observed in kidney, liver and gill tissues. Similar observations were also reported by other researchers (Dias *et al.,* 2016; Abdelhamed *et al.,* 2017; Alyahya *et al.,* 2018) in other fish species. As per earlier studies, kidney and liver are the major target organs during acute phase of *A. hydrophila* infection (Stratev *et al.,* 2015) and degenerative histopathological changes can also occur in spleen, intestine and gill. Histopathological changes associated with *A. hydrophila* infection are known to be caused by toxins and various extracellular enzymes such as protease, amylase, elastase, lipase and haemolysins secreted by *A. hydrophila* (Affifi *et al.,* 2000), whereas haemocytes and lymphocytes infiltration shows sign of host immune response and resistance towards bacterial infection.

Liver

Normal organisation of polygonal hepatic cells and blood capillaries was observed in control group. Sequential examination of histopathological changes following *A. hydrophila* infection in magur liver showed altered architecture of hepatocytes with eccentric and pyknotic nucleus, a few melano-macrophage centres (MMC) and vacuolated hepatocytes at 24 hpi. Dilated sinusoids with lipid vacuoles, vacuolated hepatocytes, blood congestion in the liver sinusoids and macrovesicular steatosis (fatty liver disease) were observed at 72 hpi. Degeneration of hepatocytes, pyknotic nuclei and MMC seen over section at 144 hpi (Fig. 4a-d). Liver specific alterations such as vacuolated hepatocytes were reported in *A. hydrophila* infected tilapia (Alyahya *et al.,* 2018); congestion in goldfish (Rosidah *et al.,* 2020); MMC accumulation in Nile tilapia (Roy *et al.,* 2018) as well as MMC accumulation, hepatocyte degeneration and vacuolation in golden mahseer (Kumar *et al.,* 2016).

Kidney

Normal organisation of kidney with many glomeruli, renal tubules and areas of hematopoietic tissues were observed in the control group. Kidney tissue in challenged magur showed degeneration in the tubular epithelium, congested glomeruli and MMC accumulation at 24 hpi. Diffuse necrosis of renal tubular epithelial cells, hyaline droplet accumulation, infiltration of lymphocytes, MMC and karyolytic nuclei were observed at 72 hpi. Degenerated tubular epithelium, heavy hyaline droplet accumulation in tubular epithelium and infiltration of lymphocytes were observed at 144 hpi (Fig. 5a-d). Kidney specific alteration such as hyaline droplet accumulation, lymphocytes infiltration and diffuse necrosis in channel catfish (Abdelhamid *et al.,* 2017); diffuse necrosis in Nile tilapia (Yardimci and Aydin, 2011) and MMC accumulation in gold fish kidney (Rosidah *et al.,* 2020) infected with *A. hydrophila,* have been reported.

Gill

Normal organisation of primary and secondary gill lamellae was observed in the control group. Infected magur gill showed hyperplasia on the tip of secondary lamellae (clubbed lamellae), hyperemia, area of inflammatory exudates, swelling of lamellae and

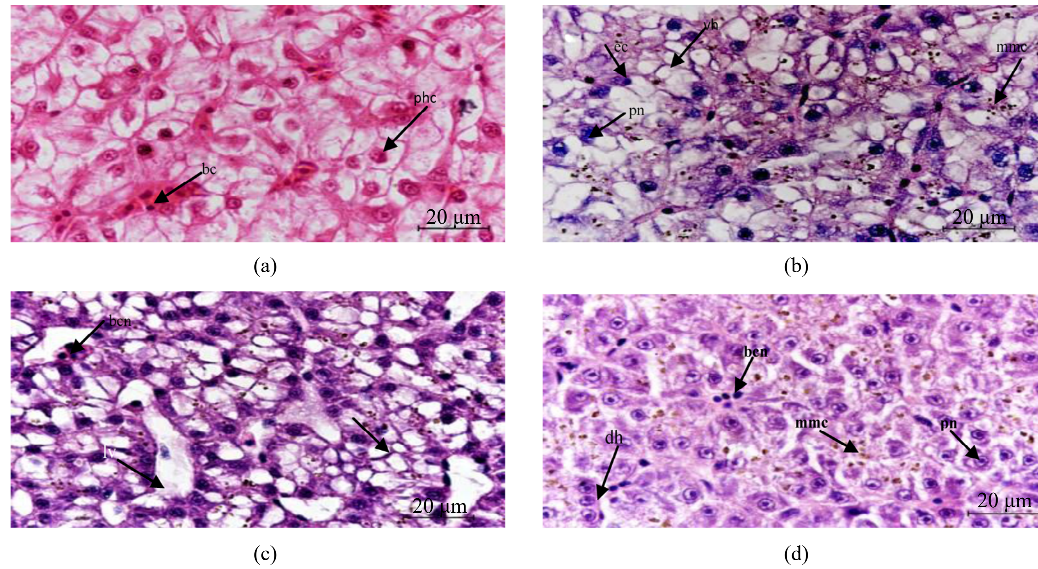

Fig. 4. Photomicrographs of histological sections of liver in control and *A. hydrophila* infected magur. (a) Liver showing normal organisation of polygonal hepatic cells (phc) and blood capillaries (bc); (b) Altered architecture, eccentric nucleus of hepatocytes (ec), pyknotic nucleus (pn), few melano macrophage centres (mmc) and vacuolated hepatocytes (vh) at 24 hpi; (c) Severe dilated sinusoids with lipid vacuoles (lv), blood congestion (bcn), macrovesicular steatosis at 72 hpi; (d) Degeneration of hepatocytes (dh), pyknotic nuclei (pn), blood congestion and mmc seen over section at 144 hpi (H&E; X100)

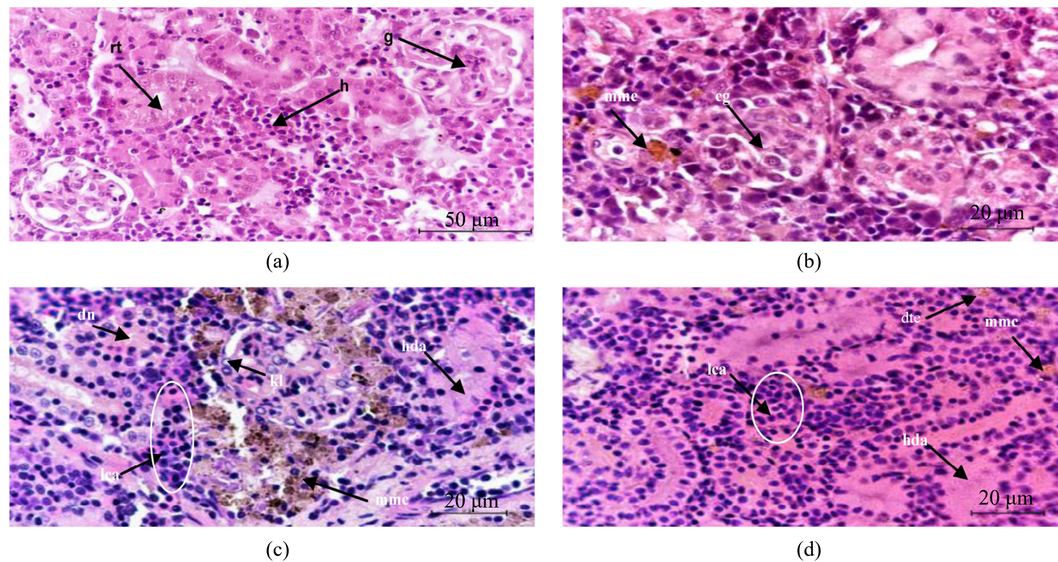

Fig. 5. Photomicrographs of posterior kidney sections of control and *A. hydrophila* infected magur (a) Normal organisation of posterior kidney with many renal tubules (rt) and area of hematopoietic tissue in between (h), glomerulus (g) (H&E; X100); (b) Mild degeneration in the tubular epithelium (dte) and congested glomerulus (cg), few MMC (H&E; X200); (c) Diffuse necrosis (dn) of renal tubules, karyolysis (kl) of its nucleus, hyaline droplet accumulation (hda), infiltration of lymphocytes (lca) and MMC at 72 hpi (H&E; X100); (d) Hyaline droplet accumulation in tubular epithelium and infiltration of lymphocytes, MMC at 144 hpi (H&E; X100)

haemocytic infiltration on the periphery of blood vessels at 24 hpi. Unilateral fusion of secondary lamellae, dilation of central venous sinus (CVS), enlarged nuclei of lamellar and filament epithelial cells and slight protrusion of filament epithelium between lamellae were also exhibited at 72 hpi. Lymphocytic infiltration, dilated CVS as well as area of spongiosis (intercellular space) in epithelium were observed at 144 hpi in gills (Fig. 6a-d). Similar lesions have also been reported in channel catfish (Abdelhamid *et al.,* 2017) and goldfish (Rosidah *et al.,* 2020).

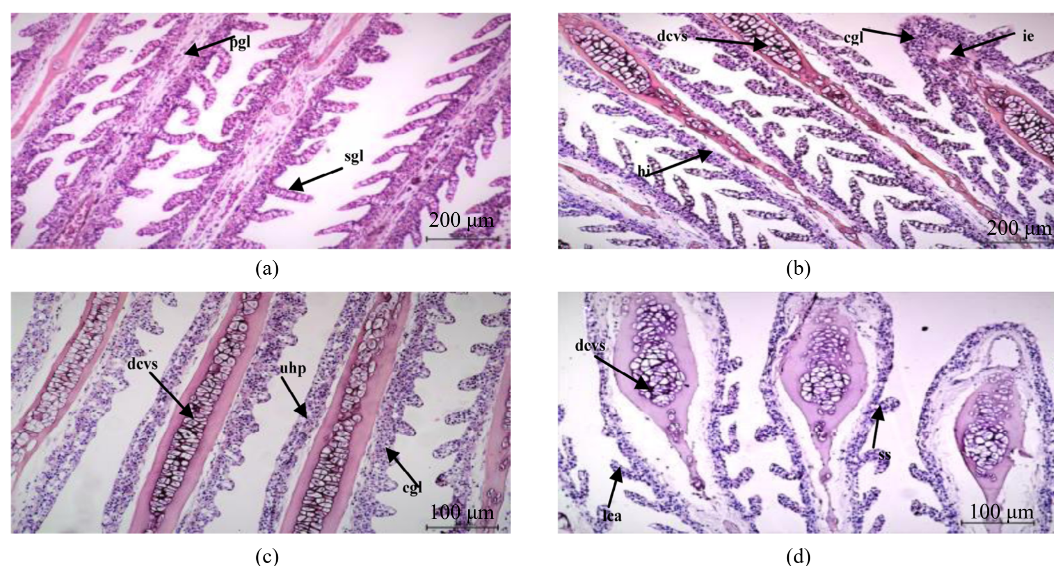

Fig. 6. Photomicrographs of gill of control and *A. hydrophila* infected magur. (a) Normal organisation of gill showing normal primary (pgl) and secondary gill lamellae (sgl); (b) Hyperemia, hyperplasia on the tip of secondary lamellae or clubbed gill lamellae (cgl), haemocytic infiltration (hi) on periphery of blood vessels, area of inflammatory exudates (ie) and dilation of central venous sinus (dCVS) at 24 hpi; (c) Unilateral hyperplasia (uhp) of secondary gill lamella, dilated CVS, clubbed gill lamellae at 72 hpi (D) infiltration of lymphocytes (lca), area of spongiosis or intercellular space (ss) in epithelium at 144 hpi (H&E; X100).

The results of the study clearly showed that most of the innate immune parameters of *C. magur* increases significantly in response to *A.hydrophila* infection. The study also revealed that *A. hydrophila* infection in magur causes degenerative histopathological changes in kidney, liver and gills. The information generated in this study would be useful to understand host-pathogen interaction and to formulate strategies for improving resistance to *A. hydrophila* infection by stimulating innate immunity. However, factors which were not analysed in the study, which could be responsible for immune protection of host need to be further investigated.

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References

Abdelhamed, H., Ibrahim, I., Baumgartner, W., Lawrence, M. L. and Karsi, A. 2017. Characterisation of histopathological and ultrastructural changes in channel catfish experimentally infected with virulent *Aeromonas hydrophila*. *Front. Microbiol.,* 8: 1519. doi: 10.3389/fmicb.2017.01519.

Afifi, S. H., Al-Thobiati, S. and Hazaa, M. S. 2000. Bacteriological and histopathological studies on *Aeromonas hydrophila* infection of Nile tilapia (*Oreochromis niloticus*) from fish farms in Saudi Arabia. *Assiut Vet. Med. J.*, 42: 195-205.

Alagappan, K. M., Deivasigamani, B., Kumaran, S. and Sakthivel, M. 2009. Histopathological alterations in estuarine catfish (*Arius maculatus*; Thunberg, 1792) due to *Aeronomas hydrophila* infection.*World J. Fish Mar. Sci.,* 1(3): 185-189.

AlYahya, S. A., Ameen, F., Al-Niaeem, K. S., Al-Saadi, B. A., Hadi, S. and Mostafa, A. A. 2018. Histopathological studies of experimental *Aeromonas hydrophila* infection in blue tilapia, *Oreochromis aureus. Saudi J. Biol. Sci.*, 25: 182-185. doi: 10.1016/j.sjbs.2017.10.019.

Anderson, D. P. and Siwicki, A. K. 1995. Basic haematology and serology for fish health programs. *Diseases in Asian aquaculture II,* Fish Health Section, Asian Fisheries Society, Manila, Philippines, p. 185-202.

APHA 1998. *Standard methods for the examination of water and wastewater,* 20th edn. American Public Health Association, Washington, DC, USA.

Aranishi, F., Mano, N. and Hirose, H. 1998. Fluorescence localisation of epidermal cathepsins L and B in the Japanese eel. *Fish. Physiol. Biochem.*, 19(3): 205-209. DOI:10.1023/A:1007779600183.

Ascencio, F., Aleljung, P. and Wadstrom, T. 1990. Particle agglutination assays to identify fibronectin and collagen cell surface receptors and lectins in *Aeromonas* and *Vibrio* species. *Appl. Environ. Microbiol*., 56(6): 1926-1931.

Banfield, M. J. and Kamoun, S. 2013. Hooked and cooked: A fish killer genome exposed. *PLoS Genet.*, 9(6). e1003590. http://dx.doi.org/10.1371/journal.pgen.1003590.

Baumgartner, W. A., Ford, L. and Hanson, L. 2017. Lesions caused by virulent *Aeromonas hydrophila* in farmed catfish (*Ictalurus punctatus* and *I. punctatus* × *I. furcatus*) in Mississippi. *J. Vet. Diagn. Invest.,* 29(5): 747-751. doi:10. 1177/1040638717708584.

Bradford, M. M. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Anal. Biochem.,* 72: 248-54. doi: 10.1006/abio.1976.9999.

Biller-Takahashi, J. D., Takahashi, L. S., Saita, M. V., Gimbo, R. Y. and Urbinati, E. C. 2013. Leukocytes respiratory burst activity as indicator of innate immunity of pacu *Piaractus mesopotamicus*. *Braz. J. Biol.*, 73(2): 425-429. doi: 10.1590/S1519-69842013000200026.

Charlie-Silva, I., Klein, A. and Gomes, J. M. M. 2019. Acute-phase proteins during inflammatory reaction by bacterial infection: Fish-model. *Sci. Rep.,* 9: 4776.

Chena, D. D., Lia, J. H., Yaoa, Y. Y. and Yong-An Zhanga, Y. A. 2019. *Aeromonas hydrophila* suppresses complement pathways via degradation of complement C3 in bony fish by metalloprotease. *Fish Shellfish Immunol.*, 94: 739-745. doi: 10.1016/j.fsi.2019.09.057.

Das, A., Sahoo, P. K., Mohanty, B. R. and Jena, J. K. 2011. Pathophysiology of experimental *Aeromonas hydrophila* infection in *Puntius sarana*: early changes in blood and aspects of the innate immune-related gene expression in survivors. *Vet. Immunol. Immunopathol.*, 142: 207-218.

Doan, H. V. 2013. The LD_{50} of Asian catfish (*Pangasius bocourti*, Sauvage 1870) challenge to pathogen *Aeromonas hydrophila* FW52 strain. *Pen. J.*, 75: 287-293.

Doumas, B. T., Watson, W. A. and Biggs, H. G. 1971. Albumin standard and measurement of serum albumin with bromocresol blue green. *Clin. Chem. Acta*, 258: 21-30. doi: 10.1016/s0009-8981(96)06447-9.

Dias, M. K., Sampaio, L. S., Proietti-Junior, A. A., Yoshioka, E. T., Rodrigues, D. P., Rodriguez, A. F., Ribeiro, R. A., Faria, F. S., Ozorio, R. O. and Tavares-Dias, M. 2016. Lethal dose and clinical signs of *Aeromonas hydrophila* in *Arapaima gigas* (Arapaimidae), the giant fish from Amazon. *Vet. Microbiol.*, 188: 12-15. doi: 10.1016/j.vetmic.2016. 04.001.

Ellis, A. E. 1990. Lysozyme assays. In: Stolen, J. S., Fletcher, T. C., Anderson, D. P., Roberson, B. S. and van Muiswinkel, W. B. (Eds.), *Techniques in fish immunology, vol. 1.* SOS Publications, Fair Haven, New Jersey, USA, p. 101-103.

Guardiola, F. A., Cuesta, A., Arizcun, M., Meseguer, J. and Esteban, M. A. 2014. Comparative skin mucus and serum humoral defence mechanisms in the teleost gilthead seabream (*Sparus aurata*). *Fish Shellfish Immunol.*, 36: 545-551. doi: 10.1016/j.fsi.2014.01.001.

Harikrishnan, R., Rani, M. N. and Balasundaram, C. 2003. Hematological and biochemical parameters in common carp, *Cyprinus carpio,* following herbal treatment for *Aeromonas hydrophila* infection. *Aquaculture,* 221: 41-50. https://doi.org/10.1016/S0044-8486(03)00023-1.

Jothi, G. E., Deivasigamani, G. B., Priyadarshini, P., Kumaran, S., Balamurugan, S., Rajasekar, T. and Sakthivel, M. 2012. Immune response against *Aeromonas hydrophila* in estuarine catfish (*Mystus gulio*, Hamilton 1822) of Kollidam Estuary: An assessment on non-specific immune parameters. *Int. J. Future Biotechnol.*, 2: 1-7.

Jiang, N., Fan, Y. and Zhou, Y. 2018. Transcriptome analysis of *Aeromonas hydrophila* infected hybrid sturgeon (*Huso dauricus×Acipenser schrenckii*). *Sci. Rep.*, 8: 17925. DOI: 10.1038/s41598-018-36376-2.

Kajita, Y., Sakai, M., Atsuta, S. and Kobayashi, M. 1990. The immunomodulatory effects of levamisole on rainbow trout, *Oncorhynchus mykiss*. *Fish Pathol.*, 25: 93-98.

Kumar, R., Pande, V., Singh, L., Sharma, L. and Saxena, N. 2016. Pathological findings of experimental *Aeromonas hydrophila* infection in golden mahseer (*Tor putitora*). *Fish. Aquac. J.*, 7: 160. doi:10.4172/2150-3508.1000160.

Magnadottir, B. 2006. Innate immunity of fish (overview). *Fish Shellfish Immunol.*, 20: 137-51. doi: 10.1016/j.fsi.2004. 09.006.

Muduli, C., Tripathi, G., Prasad, K. P., Kumar, K., Singh, R. K. and Rathore, G. 2020. Virulence potential of *Aeromonas hydrophila* isolated from apparently healthy freshwater food fish. *Biologia*, DOI 10.2478/s11756-020-00639-z.

Pal, S., Ray, S. D. and Homechaudhuri, S. 2016. Serum bactericidal activity as indicator of innate immunity in *Labeo rohita* (Hamilton, 1822) challenged with *Aeromonas hydrophila* as biomarker for clinical monitoring. *Int. J. Adv. Res. Biol. Sci.,* 3(1): 134-144.

Priyadarshini, S. K., Subramani, P. A. and Michael, R. D. 2017. Modulation of the innate immune responses in the striped snakehead murrel, *Channa striata* upon experimental infection with live and heat killed *Aeromonas hydrophila*. *Open Vet. J.,* 7(2): 157-164. doi: 10.4314/ovj.v7i2.13.

Quade, M. J. and Roth, J. A. 1997. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. *Vet. Immunol. Immunopathol.*, 58: 239-48. https: //doi.org/10.1016/S0165-2427(97)00048-2.

Rodriguez, I., Novoa, B. and Figueras, A. 2008. Immune response of zebrafish (*Danio rerio*) against a newly isolated bacterial pathogen *Aeromonas hydrophila*. *Fish Shellfish Immunol.*, 25: 239-249. doi: 10.1016/j.fsi.2008.05.002.

Rosidah, R., Yunita, M. D., Nurruhwati, I. and Rizal, A. 2020. Histopathological changes in gold fish (*Carassius auratus* (Linnaeus, 1758) infected by *Aeromonas hydrophila* bacteria with various densities. *World Scientific News,* 142: 150-168.

Roy, A., Singha, J. and Abraham, T. J. 2018. Histopathology of *Aeromonas caviae* Infection in challenged Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758). *Int. J. Aquac.*, 8: 20. DOI:10.5376/ija.2018.08.0020.

Sahoo, P. K., Rauta, P. R. and Mohanty, B. R.2011. Selection for improved resistance to *Aeromonas hydrophila* in Indian major carp *Labeo rohita*: survival and innate immune responses in first generation of resistant and susceptible lines. *Fish Shellfish Immunol.,* 31(3): 432-438. doi:10.1016/j.fsi.2011.06.014.

Sahoo, P. K. and Mukherjee, S. C. 2002. Influence of high dietary a-tocopherol on specific immune response, non-specific resistance factors and disease resistance of healthy and aflatoxin B1-induced immunocompromised Indian major carp, *Labeo rohita* (Hamilton). *Aqua. Nutr.,* 8: 159-169. DOI:10.1046/j.1365-2095.2002.00189.x.

Sharma, A., Chanu, T. I., Ande, M. P. and Jahageerdar, S. 2018. Common diseases and aberration in walking Catfish (*Clarias magur*, Hamilton, 1822) and related advisories. *Aquaculture Times*, 4(4): 14-20.

Sinha, M., Mahapatra, B. K., Saha, D. and Maitra, N. J. 2014. Mass scale seed production of magur, *Clarias batrachus* at farm level through improvised modifications. *Int. J. Fish. Aquat. Stud.*, 2(2): 210-214.

Stratev, D., Stoev, S., Vashin, I. and Daskalov, H. 2015. Some varieties of pathological changes in expimental infection of carps (*Cyprinus carpio*) with *Aeromonas hydrophila*. *J. Aquac.. Eng. Fish. Res.,* 1: 191-202.

Wiegertjes, G. F., Stet, R. J. M., Parmeatier, H. K. and Muiswinkel, W. B. V. 1996. Immunogenetics of disease resistance in fish: A comparable approach. *Dev. Comp. Immunol.,* 20(6): 365-381. doi:10.1016/s0145-305x (96)00032-8.

Xia, H., Tang, Y. and Lu, F. 2017. The effect of *Aeromonas hydrophila* infection on the non-specific immunity of blunt snout bream (*Megalobrama amblycephala*). *Cent. Eur. J. Immunol.,* 42(3): 239-243. doi: 10.5114/ceji.2017.70965.

Yardimci, B. and Aydin, Y. 2011. Pathological findings of experimental *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*). *J. Fac. Vet. Med*., 58: 47-54.

Zuo, X. and Woo, P. T. K. 1997. Natural anti-proteases in rainbow trout, *Oncorhynchus mykiss* and brook charr, *Salvelinus fontinalis* and the *in vitro* neutralisation of fish α2-macroglobulin by the metalloprotease from the pathogenic haemofagellate, *Cryptobia salmositic*. *Parasitology*, 114: 375-381. doi: 10.1017/s0031182096008578.

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