JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Histopathological changes induced in Cirrhinus mrigala due to Aeromonas hydrophilla

Sabiha Khan[1], Sonia Patharia[*2]

[1] Department of zoology, Samrat Prithviraj Chouhan Government College, Ajmer, Rajasthan, India.

[*2] Department of zoology, Samrat Prithviraj Chouhan Government College, Ajmer, Rajasthan, India.

Abstract

The aquatic environment is affected by different types of bacteria, which cause many types of diseases. *Aeromonas hydrophilla* causes a disease known as Motile Aeromonas Septicemia [MAS], which affects aquatic environments. MAS is responsible for the mass mortality of aquaculture species. Our aim was to determine the histopathological changes in *Cirrhinus mrigala* (Indian major carp fish) following *Aeromonas hydrophilla* induction. *Aeromonas hydrophila* was detected, isolated and identified from infected fish obtained from Anasagar Lake situated in Ajmer, Rajasthan (India). Fish showed reddish scar near ventral surface, fin rot, pale body color, external haemorrhages. For the detection of *A.hydrophila* BactAlert 3D 60 System was used. Blood sample from diseased fish inoculated onto the BactAlert culture media. MacConkey agar and Ship blood agar used for isolation of *A. hydrophila.* Bacteria finally identified by their specific morphological and biochemical characteristics. Further characteristics of the isolate were conducted using the vitek 2 compect system. One Year old C. mrigal carps obtained from the Aquaculture Farm. After acclimatization 4 culture tanks containing 100 L aerated water were setup and divided in two groups (G1 and G2), G1 group for control and G2 group for experimental (infected with A. hydrophila). The study of histopathological changes in C. mrigala were observe for 20, 40 and 60 days. Fishes in group G2 were injected with *A. hydrophila* isolate which grown over night on MacConey agar at 37°C. Fishes of G2 group injected an intraperitoneally with 0.1ml concentration of 10^8 CFU/ml of A. hydrophila isolates. Control (G1 group) fishes injected with 0.1ml of saline. Experimental infected fishes showed clinical signs of weakness, septicemia, anorexia, hyperemia, and lysis of the fins. Fluid accumulation was visible in abdomen and swelling in internal organs.

Keywords: *Aeromonas hydrophilla*, Blood agar, *Cirrhinus mrigala*, Histopathology, Intraperitoneally, MacConkey agar,

1. Introduction:

Cirrhinus mrigala is a species of ray-fined fish in the genus Cirrhinus and Indian major carp species cultivated widely in Southeast Asian countries. It is mainly found in freshwater in northern India, Punjab, West Bengal, and Orissa. It resembles very much with *Labeo rohita*, except that it has a wider mouth and thinner lips. The body is dark grey on the dorsal side and white-orange on the ventral side. It is a bottom feeder. It has significant economic value that is quite important.

A. hydrophila can *Aeromonas hydrophila* is a freshwater, facultative anaerobic, chemo-organo heterotrophic bacterium that causes disease in fishes, amphibians, reptiles, birds and mammals with gastroenteritis, septicemia and necrotizing fasciitis being the most prevalent kinds of disease. It can be found in various environments such as marine, freshwater, brackish water, water supplies, including sediment, estuaries,

seaweed, sea grass, used water, drinking water and food and incredibly abundant during the warmer seasons. The hosts of A. hydrophila are vast, ranging from freshwater to marine aquatic species.

Genus Aeromonas comprises Gram-negative, motile bacilli or coccobacilli rods, non-spore-forming with rounded ends that size 1–3.5 μm across and belongs to the Aeromonadaceae family of Gammaproteobacteria. They are facultative anaerobic, catalase, oxidase, and indole positive, can convert nitrate to nitrite, and are generally resistant to vibrio static agents.

A. hydrophila is an opportunistic pathogen associated with secondary infections and outbreaks. Fish infected with MAS exhibit symptoms such as loss of appetite, skin ulcerations, pale gills, swollen abdomen, and abnormal swimming patterns. *A. hydrophila* can cause MAS, which has detrimental impacts on aquaculture, such as devastating fish farms, causing economic losses, and posing a threat to public health and environmental safety. Many studies have reported that MAS is responsible for the mass mortality of aquaculture species. Furthermore, the total loss of this outbreak in commercial fish farms amounted to millions of USD, leading to the closure of numerous fish farms. MAS also alters the appearance of aquaculture species, contributing to losses in market value. Highly virulent microorganisms may cause mass mortality of aquaculture species at farm sites. Aquatic animals infected with MAS exhibit symptoms such as cloacal hemorrhage, ascites, gastroenteritic hemorrhage, and septicemia ulceration on the skin.

2. Material and Method:

The materials used for this study were *Cirrhinus mrigal* as a test fish, *Aeromonas hydrophila* isolates, MacConkeys agar, blood agar, absolute ethanol, 90% ethanol, 80% ethanol, xylol, hematoxylin eosin (HE), aquades, and clinical syringes.

Previously, *Aeromonas hydrophila* isolated from diseased fish collected from Anasagar Lake situated in Ajmer, Rajasthan (India) were subcultured onto MCA plates (**Griffin et al. 2013**) and incubated at 37C (**Abbott., 2010**). The morphological characteristics of the bacterial colonies, including shape, size, and color, were investigated on MCA plates. Simultaneously, physiological characteristics were studied by observing the growth of isolated colonies at various temperatures (4, 37, and 40 °C). The biochemical identification of the isolates performed using the Gram staining, motility, oxidase, and catalase tests (Table 1). Further characterization of the isolates was conducted using the VITEK 2 compect system (Table 2) **(Ramalivhana J. N., et al., 2009)**. *Cirrhinus mrigala* were collected from a Aquaculture farm and brought in the laboratory were humanely euthanized. Fishes acclimatized in aerated water for three days. Four culture tanks containing 100 L of aerated water were setup for control, 20, 40, and 60 days for experiment. During the experiment fishes were fed daily. The water in the tank was partially exchanged for 3 to 5 days to remove waste feed and fecal matter. The water temperature (26±1oC) water pH (6.8±0.2) and oxygen concentration in the range of 5 to 6 mg/l were maintained. For the pathogenicity test, an intraperitoneal injection method was used to observe the effect of infection and pathogenicity of the pathogen for 20, 40, and 60 days. Sterile and disposable 1 ml insulin syringes were used for the injection**.** Each fish was intraperitoneally injected with 0.1 ml of 10^8 CFU/ml, and control fish were injected with 0.1 ml of saline. Fish exhibiting clinical symptoms were used for histopathological studies. Experimental infected *C. mrigal* showed clinical signs of weakness, swimming closer to the surface, septicemia, anorexia, hyperemia, and lysis of the fins. Fluid accumulation in abdomen, and swelling were visible internally in fishes. C. mrigal were monitored for 20, 40 and 60 days after being infected to study the histopathological changes in their organs. Kidney and liver samples were collected from healthy and diseased fish. During sampling, utmost care was taken to avoid contamination and human infection.

2.1 Histopathological study- Tissue specimens of the liver and kidney were obtained from control and infected fish, rinsed in normal saline, and fixed in 10% formalin buffered for 48 h separately (after changing formalin for 24 h). After fixation, they were dehydrated using an automated processor. For dehydration, the samples were kept in formalin overnight, then in pure alcohol for 6 h, then in acetone for one & half h, cleared in xylene for half h, embedded in paraffin wax (58-60o C pellets) for 12 h at 65o C temperature, and then the blocks were prepared. After freezing the blocks trimming 4-5 μm thick sections were trimmed using a microtome machine, then the sections were affixed on a glass slide by gently, left in an oven for 10 min for deparaffinization, and then stained with hematoxylin-eosin (HE). The slide was then covered with a coverslip using DPX.

3. Observation :

3.1 Bacteriological observations and morphological characteristics of isolates:

Aeromonas hydrophila identified based on morphological and biochemical analyses. Morphologically, the isolated colonies were yellowish, opaque, round, convex, smooth-edged, and semi-translucent on MacConkey agar plate. They were gram-negative and rod-shaped. The biochemical characteristics of the isolates summarize in Table 1.

Table 1: Biochemical characteristics of isolates *A. hydrophila*

Chacteristic	Response of A. hydrophilla	
Gram reaction	-	
Shape	Rod	
Motality	+	
Oxidase	+	
Catalase	+	
Glucose	+	
Lactose	+	
Indole	+	
Inositole	–	
Growth at different temp.	4°C	–
	37°C	+
	40°C	–

(+): positive response, (-): negative response

3.2 Vitac 2 compact microbial identification

Few biochemical characteristics were determined using the VITEK 2 compact system. The positive or negative reactions of the isolates are summarized in Table 2.

Table 2: Biochemical characteristics of isolates determined using the Vitek 2 compact system.

Biochemical	Response to isolate
APPA Ala-phe-pro arylamidase	+
PyrA L-pyrrolydonylarylamidase	–

TyrA Tyrosine arylamidase	+
ProA Prolenearylamidase	+
BGAL Beta galactosidase	+
H	+
BNAG Beta-N	+
dGLU D-glucose	+
SAC Sucrose	+
GGT Gamma glutamyletrasferase	–
dMAL D-maltose	+
dMAN D-mannitole	+
dMNE D-mannose	+
PHOS Phosphatase	–
dTAG D-tagatose	+
dTRE D-trehalose	+
dCEL D-cellobiose	+
OFF Fermentation	+
MNT Malonate	–
CIT	–

Citrate-sodium	
URE Urease	–
LDC Lycine decarboxylase	–
LIP Lipase	+
dSOR D-sorbitole	–
ODC Ornithine decarboxylase	–

3.3 Histopathological observation

Histopathological Observation of Liver and Kidney of *C. mrigala* were shown in fig 1 & fig 2.

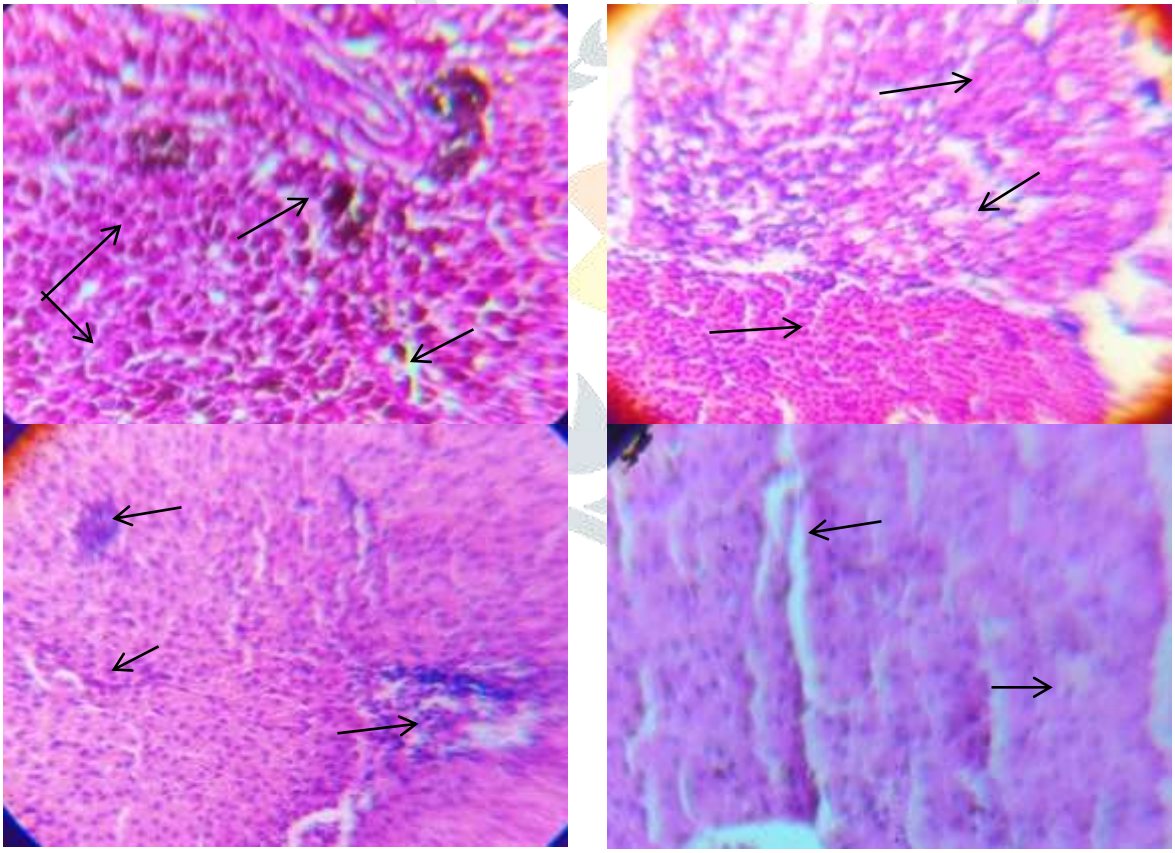

Fig 1: A to D; Photomicrograph of transverse section of liver of *Cirrhinus mrigala* showing (A) unequal size of hepatocytes, vacuolated hepatocytes, hemorrhagehage in the liver parenchyma. (B) Degeneration of hepatocytes, inflammation and incomplete epithelial cells, and necrotic hepatocytes. (C) Pycnosis of Hepatocyte nuclei, fibroblasts, inflammatory cells, and fibrosis in the central vein. (D) Dilation of blood sinusoid and Peribiliary cirrhosis

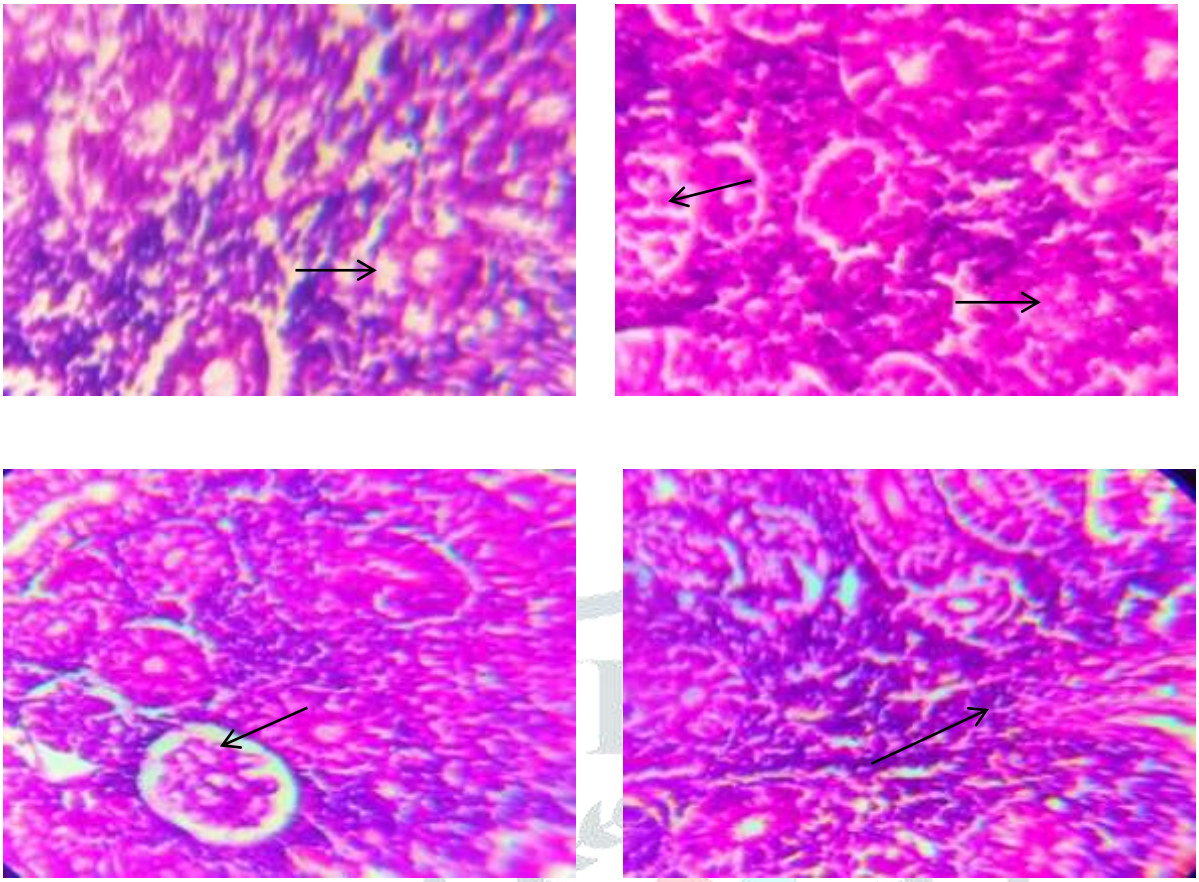

Fig 2. (A to D): Photomicrograph of transverse section of Kidney of *Cirrhinus mrigala* showing (A) Vacuolation of tubular epithelial cells. (B) Tubular necrosis. (C) Glomerular atrophy. (D) Infiltration in intertubular region.

4. Result :

4.1 Clinical Findings:

After injection, all experimental fish were observed swelling at the injected areas and almost all fishes were gather in a place. The acceptability of the feed was reduced, and darkening at the injected areas was visible, after the post-injection period. Focal hyperemia of the skin was observed over the pectoral fins. Weak fishes came closer to the surface, and 20% mortality was recorded. Food rejection, unstable swimming on the bottom of the tank, gross symptoms of the diseases such as nasty ulcerative lesions on skin with erosion of scales and fin rot, tail rot, hemorrhagic skin, and distended abdomen were notice.

4.2 Histopathological changes:

Major histopathological changes of the experimental infection in C. mrigala were mainly observed in the liver and kidney.

4.2.1 Liver: Experimentally infected fish showed generalized swelling, incomplete epithelial cells, unequal size and hypertrophy of hepatocytes, dilation of blood sinusoids, pyknotic nuclei, necrosis loss of nuclei of hepatocytes and inflammatory cells, granular degeneration, and vacuolation of hepatocytes along with the appearance of vacuoles in the hepatocytes of fish due to the release of blood cells. Peribiliary cirrhosis and infilteration of cells, cholestasis and severe steatosis

4.2.2 Kidney: Histopathological changes in the kidney were observe, chiefly vacuolation of the tubular epithelial cells and glomerular atrophy. Mild-to-moderate and strong congestion of the blood vessels with hemorrhage at certain places and atrophic changes in the glomeruli were indicated by the enlargement of the bovine space. Other changes were degeneration and desquamation of the tubular epithelium, vacuolation of the

epithelial cells and necrosis of the renal tubules. Infiltration of polymorphic nuclear cells leading to massive widening of the intertubular area was also observed.

5. Discussion

In the present study, the bacterial isolate associated with septicemic disease of carps was A. *hydrophila*. Motile aeromonads **(Cipriano et al., 2001)** cause diverse pathologic conditions, including dermal ulceration, tail or fin rot, erythrodermatitis, hemorrhagic septicemia, red sore and rot disease and scale protrusion disease. The gross signs of disease after infection in fish include abdominal distension, focal hemorrhagic necrosis, dropsyopsy (**Grizzle et al.,1993)**. The target organs for acute A. hydrophila infection in fish are mainly found in the liver and kidney **(Khan S. et al., 2017)**. Rupture of congested portal vessel, necrosis, pycnosis, vacuolation of hepatocytes and epithelial cells, glomerular atrophy, and degeneration of tubular epithelium were observed in the liver and kidney of *C. mrigala*.

6. Conclusion

This study suggests that freshwater fishes could always be under the threat of *Aeromonas* infection, since the species are common in freshwater environments. *Aeromonas* spp. might also pose a threat to public health, especially to people who come into contact with diseased fish. The study clearly shows that typical histopathological alterations seen in the functional epithelium of the liver and kidney. We hypothesized that the bacterium could be one of the major pathogens of carp farming. However, further studies are needed to demonstrate the molecular and serological characteristic of the organism. In addition, identification of virulence factors of *A. hydrophila* from carp also needs to be studied. An infection of *Aeromonas hydrophila* ultimately lead to the high mortality of fishes. Therefore, the scope of this study is the proper management of water quality, food health and preventing and controlling *A. hydrophila* infection in fish are required and avoiding its transmission to humans.

7. Acknowledgement :

I would like to thank Maharishi Dayanand Saraswati University, Ajmer for granting the Ph.D research work. The Department of Zoology, Prithviraj Chouhan Government College, Ajmer of the M D S University is highly appreciated for allowing the laboratory work. I am also grateful to the Microbiology Department of J L N Medical College Ajmer for laboratory work and also thank to Dr.Sabiha Khan from Department of Zoology S P C Government College Ajmer for her guidance in research.

Author Contributions

Dr. Sabiha Khan : Gave her guidance in paper.

Sonia Patharia : Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from commercial and financial funding agencies.

Data availability statement

Data included in article/ supplementary material/ referenced in article.

Informed consent statement

This study did not involve human participants therefore informed consent was not required.

Clinical trial registration

This research does not involve any clinical trial.

Permission to reproduce material from other sources

Not applicable.

Ethics statement

Not required.

Additional information

No additional information is available for this paper.

Conflict of interest

The authors declare no conflict of interest.

References

1. Abbott S. L., Janda J. M. The genus Aeromonas: taxonomy, pathogenicity, and infection. Clin.Microbiol. 2010; Rev., 23(1): 35-73.Doi: 10.1128/CMR.00039-09.
2. Ahammed T., Borty S. C. Monir M. S. et al. Isolation, identification and molecular detection of Aeromonas hydrophila from diseased stinging catfish Shing (Heteropneustesfossilis).Asian Australas. J. Biosci. Biotechnol. 2016; 1(1): 125-133.
   Doi: https://doi.org/10.3329/aajbb.v1i1.61545
3. Cipriano C., Rocco. Aeromonas hydrophila and Motile Aeromonas Septicemias of fish. Fish disease leaflet. 2001;68.
4. Grrifin M. J. et.al. Quantitative detection of Aeromonas hydrophila strains associated with disease outbreaks in catfish aquaculture. 2013; 25(4) 473-481. Doi: 10. 1177/1040638713494210.
5. Grizzle J. M., Kiryu Y. Histopathology of gills, liver, pancreas and serum enzyme level of channel catfish infected with A. Hydrophila complex. Journal of aquatic animal health. 1993; 5: 36-50. Doi: 10.1577/1548-8667(1993)005<0036:hoglap>2.3.co;2
6. Koca C., Sarimehmetoglu B. Isolation and identification of motile Aeromonas spp. in turkey meat. Ankara University, Vet. Fak. Derg. 2009; 56(2): 95-98.bsarimeh@veterinary.ankara. Edu. tr
7. Khan S., et al. Histopathological Biomarkers as Indicators of Aquatic Pollution, World Journal of Pharmacy and Pharmaceutical Sciences, 2017; Volume 6, Issue 13, 703-709., ISSN 2277– 7105.
8. Ramalivhana J. M., Obi C. L., Moyo S. R. Antimicrobial susceptibility testing of A. Hydrophila isolated from Limpopo province, South Africa using VITEK 2 system, micro scan walk away, Disc diffusion and E-test method, African journal of microbiology research, 2009; 3(12): 903-913. http://www.acadmicjounrnals.org/ajmr. ISSN 1996-0808©2009 Acadmic journals.
9. Sharma I., Kumar A. Pramanik A.K. Isolation and identification of mesophillic Aeromonas bacteria from meat and fish foods of North East India. 2009; J. Pure Appl. Microbiol.,3(2): 517-526. gduttasharma@gmail.com