IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN (P): 2347–4580; ISSN (E): 2321–8851
Vol. 9, Issue 3, Mar 2021, 29–36

HISTOPATHOLOGICAL CHANGES IN THE SKIN AND GILLS OF CHANNA PUNCTATUS DUE TO BACTERICAL INFESTATION CAUSES EPIZOOTIC ULCERATIVE SYNDROME (EUS)

Podeti. Koteshwar Rao

Research Scholar, Department of Zoology, Kakatiya University, Warangal, Telangana, India

Received: 08 Mar 2021 **Accepted:** 17 Mar 2021 **Published:** 31 Mar 2021

ABSTRACT

The present study was carried out to study the skin and gills histopathology in the freshwater fish, Channa punctatus which is exposed to bacterial infestation. Different histopathological changes were observed in fish organs. Histopathological changes in skin and gills observed microscopically showed increased degrees of damage in the tissues in correlation with the concentration of polluted toxic substances while skin and gills of control groups exhibited a normal architecture. The seasonal variation of skin and gill pathology of Channa punctatus was carried out from two lakes of Hasanparthy and Bhandam in Warangal, Telangana, India during the months of Decmber to February 2019. For the histopathological studies of skin and gill were collected processed and stained with Azan. Histologically the Channa punctatus of two lakes of fishes were more affected in winter months. The investigated organs of Murrels were exhibited some abnormalities during winter however mild hyperplasia, haemorrhage, partial loss of secondary gill lamellae, marked and hypertrophy was observed. Several pathological changes like necrosis, fungal granuloma, protozoan and cyst, vacuolation, melanomacrophase, haemorrhage, hypertrophy, hyperplasia and clubbing were recorded in all the investigated organs. Large bacterial colony and protozoan cyst were observed in the secondary gill lamellae of Channa punctatus.

KEYWORDS: *Channa Punctatus, Hasanparthy, Haemorrhage*

INTRODUCTION

Histopathology refers to the branch of pathology which deals with the diagnosis of tissue. Even today, disease manifestations based on histological sections stained with different stains form the most important tool in disease diagnosis. (Dutta, 1996). Histopathology is able to evaluate the early effects and the responses to diseases. Environmental factors and poor water quality resulting from increased pollution due to effluents discharge and pathogen transfer appear to be as important underlying cause of epizootics. EUS is one of the most destructive diseases among freshwater as well as brackish water fish species in the Asian Pacific region (Muthu Ramakrishnan et al.,2015). The present study purposes at a preliminary exploration of the possible sources of Aeromonas hydropyhyla, Staphylococcus aureus that may result in infection of Channa punctatus. Therefore, the study of bacterial and parasitic infections causing red spot disease (bacterial hemorrhagic septicemia) on this Channa fish of commercial importance has been considered. The objective of the present study is to elucidate the structural deformity of skin and gills of the freshwater fishes caused by Aeromonas hydrophila Staphylococcus aureus species. The epizootic ulcerative syndrome (EUS) resulted in Channa spp, Puntius spp,Anabas sp. and other indigenous fish species being seriously affected. External parasites infection of the skin and gill is a common problem of fish health under aquaculture farming conditions. (D. R. Das et., al 2018).

This work applied on Channa punctatus to investigate some bacteria causes' skin ulceration. Skin acts as a mirror to the health state of the fish, since some pathogens attack the skin not only due to surface contamination but also due to invasion by pathogenic microorganisms. Some of these pathogens that isolated from the skin and gill in Channa punctatus are Aeromonas sp, Pseudomonas sp. Red spot disease has been recorded as the most common disease.

Histopathological analysis of the skin and gills of C. punctatus were performed to detect the pollution impact of an environmental contamination and bacterial infection. So that the gills can be used as efficient bio indicator. Fish gills function as both respiratory and excretory organs. Basically they consist of a network of capillaries where blood is separated from the surrounding water by only one or two layers of cells. Proliferation of epithelial tissue, and later the loss of surface by the clubbing and fusing of lamellae, impair respiration and the excretion of nitrogenous waste materials, and disturb osmotic balance. Because these changes adversely affect the health of fish, the prevention and treatment of gill diseases are important in fish culture.

MATERIAL AND METHODS

The specimens were deeply anaesthetized by immersion into 5 ml/L aqueous solution of ethylene glycolmonophenylether. After dissection of fishes, sample tissues of skin and gills were carefully removed and small pieces were preserved in fixatives. Tissues of skin and gills were preserved in Bouin's, Zenker's, Susa, Carnoy and Formal Calcium standard fixatives as per protocols. The infected fishes were identified by red spots on their body, excess mucus secretions, damaged and sluggishness. The Microbial investigation was carried out in different infected parts were separated from fish of Channa punctatus namely, skin and gills. The bacterial microorganisms such as Aeromonas hydropila, Staphylococcus aureus, determined in above the organs. (Figure 1 & 2) Control and infected Channa punctatus.

Figure 1: Control Channa Punctatus.

Figure 2: Infected Channa Punctatus.

Processing for Histopathological Investigations

Samples of skin and gills tissues were taken out of fixative.

- **Hydration:** Washing of these tissues was done with saline water/solutions as per procedures adapted.
- **Dehydration:** The removal of water or dehydration was done by' transferring tissue into the 70 % alcohol of sufficient quantity and then graded alcohol up to 90 % for one change and two changes of absolute alcohol.
- **Clearing:** The clearing of tissues was done by subjecting to a clearing agent i.e. xylene.

- **Embedding and Impregnating:** The impregnation agent used was paraffin wax, as it facilitated easy penetration into the tissue without causing structural damage and without much shrinkageor crystal formation.
- **Trimming:** The embedded block containing tissue was further trimmed such that only one tissue is subject for cutting into sections.
- **Mounting of the Block:** An iron blocks of 30 mm/was used to hold the block at the correct angle and in position for cutting.
- **Section Cutting:** The block containing tissue was cut at 5µmthickness with the help of a microtome.
- **Floating out and Mounting of Sections:** The cutting sections were allowed to float in a water bath so as to avoid wrinkles. Further it is also seen that the cut sections were adhered to the slides firmly and in a right position. The slide was given an egg albumin coating before affixing the sections over it.
- **Staining the Slides with Azan and Mounting:** The stained sections were observed under light microscope and microphotographs were taken for pathological observations.

RESULTS

Histopathology of Skin

Histological, morbid fish often exhibit epidermal erosion infected with Aeromonas species colonization but minimal associated inflammation. In Channa punctatus loss of epidermis was wide spread over large portions of the body since then, the infection has been diagnosed. Histopathological changes of skin tissue infected with S. parasitica had shown loss of epidermis and necrotized hypodermis, muscle layer shows inflammation with necrotic tissue debris and formation of number of well developed layered granulomas surrounded by hyphae Hatai (1980) Granulomas showed fribrillar structures due to hyphal infection. A. laevis injected tissue showed more or less similar symptoms as that with S. parasitica. Necrotized epidermis and hypodermis with granulomatous response of musculature.

Histological studies of tissue injected with A. niger showed no granuloma formation in musculature, hyphae was seen in deeper layers although epidermis was degenerated and edema was observed in underlying hypodermis and musculature, (Hatai et al.,1994 and Hussian et al.,2013). Granulomatous response due to Aphanomyces infection was also reported Qureshi et al., (2001). Histopathological studies of tissue infected with A. Niger did not show any granuloma formation, however degenerated musculature was observed with necrotized hypodermis and epidermis, (Rekha Chauhan et al., 2014). The lesions had well developed granulomas that under laid in the loss of epidermis Lesions appeared to originate in the muscle or overlying subcutaneous tissue, from these spread to underlying organs. There was heavy lymphocyte infiltration as well. (Ganapathy Rameshkumar et.,al 2013).

Hemorrhage was observed within the necrosis part, small number of melanin pigment of containing cells began to be encountered under the basement membrane of the epidermis. Damaged portion of the dermis still disconnected. Musculature was clearly observed where the muscle, fibers were hyalinized with prominent destruction of the nuclei. The tissue reaction was less common where the melanophores activation was neglected. (Figure 3 & 4).

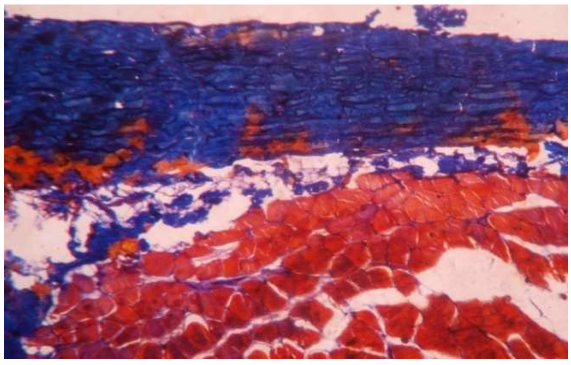

Figure 3: Control Skin of C. Punctatus.

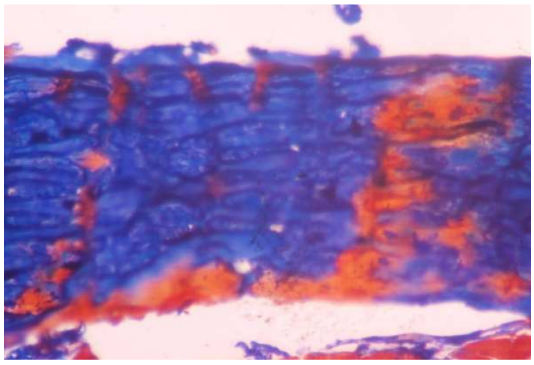

Figure 4: Infected Skin of C. Punctatus.

Histopathology of Gill

EUS infection has induced marked pathological changes in fish gills architecture. The damage was severe in gills of fishes with high level of EUS infection. Shortened and clubbing of ends of the secondary gill lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary lamellae were well marked. Hyperplasia and hypertrophy of nuclei were also seen. Besides these changes pyknotic nuclei, vacuolization and degeneration of epithelial cells and pillar cells and lifting of the epithelial layer from the secondary lamellae were also observed.

Bacterial gill disease is the most lethal of these categories, are discussed here. In the present study, hyperplasia and hypertrophy of the epithelial cells, epithelial lifting, lamellar disorganization, lamellar aneurysm, rupture of the lamellar epithelium, rupture of pillar cells and necrosis were observed due to EUS infection. Similar type of pathological changes was observed by many researchers on bacterial and fungal toxicity. De Silva and Samayawardhena (2002) observed irregular appearance of gill lamellae, increased vacuolation in epithelial cell, lamellar fusion and complete destruction of gill lamellae in poecilia reticulate exposed to chlorpyrifos.

The damages occurred in the secondary gill lamellae with light precipitation of mucous and exfoliated nuclei, splitting of muscle fibers in the freshwater fishes exposed to acute and chronic EUS infections. Bacteria and fungi affect the organ systems specifically during the winter season. histopathological changes such as cellular hypertrophy or hyperplasia in the epithelial layer of primary filaments and fusion of secondary lamellae. (A. A. Hadi et.,al 2012). Severe hyperplasia, results in the fusion of secondary lamellae frequently also results in alterations such as blood congestion, hypertrophy of epithelial cells and lamellar disorganization (Marina et al., 2007). Hyperplasia of the epithelial cells and subsequent lamellarfusion, goblet cell proliferation as well as the migration of eosinophilic granular cells (EGCs) to gills of fishes infected with these parasites has been recorded (Mehdi Raissy and Mahsa Ansari, 2011). The gill lamellae of the EUS infected Channa punctatus showed well developed encapsulated granulomas around necrotic area is observed, which appeared nodular with characteristic focal (tubercles) granulomas. Laharia Reena et.,al 2020. Gill lamallae with degraded epithelium and fungal hyphae encapsulated by multiple layer of fusion of some secondary lamellae was due to severe infection. In the months of October and November, both the primary and secondary gill lamellae were arranged systematically and no significant pathological symptoms were observed in the structure of gill. However, in December, primary gill lamellae were hypertrophied and few blood cells were accumulated at the base of secondary lamellae. Inflammatory cells and mild haemorrhages were also observed in the primary gill lamellae during this winter period in all farms. However, in the month of January, primary gill lamellae were severely affected followed by marked hypertrophy and hyperplasia and secondary gill lamellae partly missing (Chandra et al., 2012). (Figure. 5 & 6).

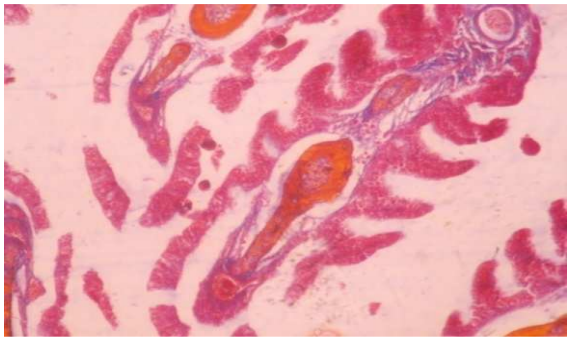

Figure 5: Control Gill of C. Punctatus.

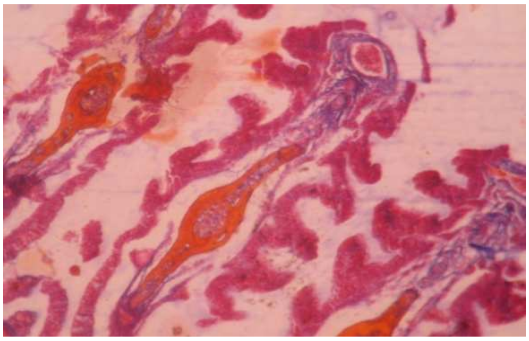

Figure6: Infected Gill of C. Punctatus.

DISCUSSIONS

In the skin of Channa punctatus, epidermis, dermis and hypodermis were partly lost and some vacoums were seen in dermis and myotomes were arranged. Dermis and muscles were severely necrotic which created huge vacuums. Observed histological differences in channel cat fish, Ictalurus punctatus (Rafinesque), with naturally occurring bacteria isolated from lesions of skin and superficial muscle and systemic Aeromonas hydraphyla infections i.e., diffuse necrosis into and several internal organs. In present study, it has been observed that morbid fish exhibited complete mucosal erosion, muscle fibers regeneration. In the regenerated muscle bundle almost replaced by fibrosis area, severe regressive changes in muscles and necrotic changes developed in muscle fibers. Hemorrhages were observed within the necrosis area small number of melanin pigment of containing cells.

The primary gill lamellae are flat leaf like structures with a central rod like supporting axis and a row of secondary gill lamellae on each side of it. They are situated laterally on either side of interbranchial septum. The primary gill lamellae consist of centrally placed rod like supporting axis with blood vessels on either side. The surface is covered with simple squamous epithelial cells separated by mucous cells. Numerous blood vessels are extended into each of the secondary gill filaments. The blood cells of the secondary gill lamellae have a single nucleus which is flat in appearance. A number of cuts were also observed in secondary gill lamellae. The pillar cell nucleus showed necrosis and developed vacuoles in the secondary gill epithelium. There is tendency of fusion of disorganized secondary gill filaments. The changes reported in the gill include epithelial proliferation, congestion of blood vessels and hyperplasia.

Histopathological changes in the gill of Labeo rohita were reported (Vijaya Lakshmi and Tilak 1996). The fish were exposed to organophosphate pesticide monocrotophos. Similar changes were also observed (Kumaraguru et al.,1982); (Jayantha Rao et al.,1985; (Sunita Rani and Due1999); (Tilak et al.,2005a) reported that chlorpyrifos intoxication in fish, Catla catla caused dropsy, vascular degeneration, cloudy swelling, necrosis and other degenerative changes in epithelial and pillar cells of the gills. Club shaped lamellae represents progressive degeneration in the gills. (Wannee et al.,2002) stated filament cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial lifting and aneurysm in the Nile tilapia, Oreochromis niloticus under exposure to glyphosate for 96 hr.

CONCLUSIONS

The skin showed focal sloughing of the epidermis with hyperplasia and mucous cells. The dead cells formed due to sarcolysis. Congested and hemorrhagic dermis with excessive aggregation was observed. Morbid fish exhibited complete mucosal erosion and necrotic changes in muscle fibers. Marked pathological changes in fish gill architecture were

observed. The changes include epithelial lifting, bulging of tips in primary gill filaments, degenerated secondary lamella, curling of secondary gill filaments, atrophy in secondary lamella and fusion of secondary gill filaments. The damage of gills in fishes exposed to the high level of disease was severe. Shortened and clubbing of ends of the secondary gill lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary lamellae were well marked. Hyperplasia and hypertrophy of nuclei were also seen.

ACKNOWLEDGEMENT

Authors with to thanks Dr. P. Srinivas Plant Pathology and Microbiology laboratory, Department of Biotehnology, Kakatiya University, Warangal and Dr. G. Rajender Department of Zoology Kakatiya University, Warangal for their continuous support and inspiration and providing necessary facilities for the work.

REFERENCES

1. *Hadi and S. F. Alwan. (2012). Histopathological changes in gills, liver and kidney of fresh water fish, Tilapia zillii, exposed to aluminum. Int. J. of Pharm. & Life Sci. (IJPLS), Vol. 3, Issue 11: November: 2071-2081 2071.*
2. *D. R. Das and K. J. Chandra. (2018). Seasonal Variation Of Gill, Skin, Muscle, Liver And Kidney Pathology Of Mrigal (Cirrhinus Cirrhosus) In Cultural Pond Fisheries, Mymensingh, Bangladesh Bangl. J. Vet. Med. 16(1): 121–129.*
3. *Dutta, H.M. (1996). In Fisz Morphology, A composite approach for evaluation of the effects of pesticides on fish, Eds Munshi JSD, Dutta HM (Science Publishers) Inc, NewYork.*
4. *Ganapathy Rameshkumar, Samuthirapandian Ravichandran. (2013). Histopathological changes in the skins and gills of some marine fishes due to parasitic isopod infestation. Journal of Coastal Life Medicine; 1(1): 12-18.*
5. *Hatai K, Nakamura K, Rha SA, Yuasa K, Wada S. (1994). Aphanomyces infection in the dwarf Gourami (Colisa lalia). Fish pathol. 29: 95-99.*
6. *Hatai, K. (1980). Studies on pathogenic agents of Saprolegniasis in fresh water fishes.Special Re Nagasaki Pref. Inst. Fish.No. 8, 95 p.*
7. *Hussian, M.M.A. Hassan W.H. and Mahmood. M.A. (2013). Pathogenicity of Achlya proliferoids and Saprolegnia diclina (Saprolegniaceae) associated with saprolegniasis outbreaks in cultured. Nile Tilapia (Oreochromis niloticus). World J. of Fish and Marine Science.5 (2); 188-193.*
8. *Jayantha Rao, K. Ch. Madhu and K. Rama murthy. (1985). Histopathological and histochemical*
9. *Changes under phosphomidon intoxication in liver of freshwater fish Tilapia mossambica. Proc. Bull. Environ. Sci. 3, 20-23.*
10. *Kumaraguru, A.K. and F.W.H. Beamish and H.W. Ferguson. (1982). Direct and circulatory paths of permethrin causing histopathological changes in the gills of rainbow trout. J. Fish. Biol. 29, 87-90.*
11. *Laharia Reena1 and Patel Paresh. (2020). Histological changes in skin and gill of fresh water EUS infected fish Channa punctatus. Environment Conservation Journal, 21 (1&2): 75-84,*

12. *Vijaya Lakshmi, S. and K. S. Tilak. (1996). Effect of pesticides on the gill morphology of Labeo rohita. J. Ecotoxicol. Environ. Monit. 6, 059-064.*
13. *Marina M.P. Camargo and Claudia B.R. Martinez. (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotrophical Ichthyology, 5 (3): 327-336.*
14. *Mehdi Raissy and Mahsa Ansari. (2011). Histopathological changes in the gills of naturallyinfected Capoeta aculeata (Cuvier and Valenciennes, 1844) with parasites. African Journal of Biotechnology Vol. 10(68), pp. 15422-15425.*
15. *Qureshi, T. A. ChauhanR. and Mastan.S. A. (2001). Histopathology of Mycoses in certain fishes of Bhopal. Indian J. Anim. Hlth. 40(1): 93-96*
16. *Rekha Chauhan, Majid Hassan Bhatt, Showkat Aziz Lone. (2014). Pathogenic Effects of Three Species of Fungi. (Aphanomyces laevis, Aspergillus niger and Saprolegnia parasitica) on Gold Fish (Carrasius auratus L). Indo Global Journal of Pharmaceutical Sciences, 4 (2): 41-46.*
17. *Sunita, Rani and K.K. Due. (1999). Cypermethrin toxicity induced histopathological and Biochemical changes in the liver of albino rats (Rattus norvegicus). J. Ecotoxicol. Environ. Monit. 9, 41-46.*
18. *Tilak, K.S., D. Koteswara Rao and K. Veeraiah. (2005a). Effects of chloropyrifos on Histopathology of the fish Catla catla. J. Ecotoxicol. Environ. Monit. 15, 127-140.*
19. *Vijaya Lakshmi, S. and K. S. Tilak. (1996). Effect of pesticides on the gill morphology of Labeo rohita. J. Ecotoxicol. Environ. Monit. 6, 059-064.*
20. *Wannee, Jiraung Koorskul, E Suchart Upatham, Maleeya Kruatrachue, Somphong Sahaphong, Suksiri Vichasri-Grams and Prayad Pokethitiyook. (2002). Histopathological effects of round up, a glyphosate herbicide, on Nile tilapia Oreochromis niloticus. Science Asia, 28, 121-127.*

HIGHLIGHTS OF THE ARTICLE

21. *This research papper explans the Bacterial diseases of Freshwater Fish of Channa punctatus*
22. *And this paper contains the histopathological study of Channa punctatus*
23. *This research paper will help to fish farmers for how control the diseases in the winter season*