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Histopathological changes in the gills of fresh water fish *Channa striatus* (Bloch) infected with Epizootic Ulcerative Syndrome (EUS)

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Abstract
Fish diseases are the vital problem of the fishery industry. Fish requires optimal hydrobiological parameters for growth and survival. Poor pond management practices, higher stocking rate and uncongenial environmental conditions result into disease outbreaks. The current study was performed on *Channa striatus* which are also known as 'Snake-heads', that are infected with Epizootic Ulcerative Syndrome (EUS). The acclimmatized fishes were classified with selective lesions as healthy and diseased fish; the latter were further classified as mildly infected, severely infected fish and their gills were examined for macroscopical and histological lesions. The Structural alterations such as change in shape, epithelial proliferation; lamellar fusion and necrosis were observed. It can be concluded from our study that structural and microscopic alterations in gills serve as biomarker for severity of EUS in fish.

Keywords: Kolleru Lake, *Channa striatus,* Epizootic Ulcerative Syndrome, Gill, Histopathology

1. Introduction
Histopathological investigations have long been recognised to be reliable biomarkers of stress in both in capture and culture fisheries [1]. Histological analysis appears to be very sensitive parameter and it is crucial in determining cellular changes that may occur in target organs such as the gills, muscle, liver, brain and kidney [2]. Histological investigation may therefore prove to be a cost effective tool to determine the health of organisms, hence reflecting the health of an entire aquatic ecosystem. Pathogens produce tissue level changes in fish such as necrosis in the liver, tubular damage in kidney acetylcholinesterase (Ach. E) activity in the brain and gill lamellar abnormalities [3]. Therefore histopathological studies are necessary for the description and evaluation of potential lesions in aquatic animals exposed to various infections and toxicants [4].

Most of the causative micro-organisms are naturally occurring saprophytes, which play a considerable role in the synthetic pathways and degradeative processes of the Aquatic environment, using the organic and mineral matters of the milieu for their growth and multiplication. It has been shown by several workers that the normal bacterial flora of fish is a direct reflection of the bacterial population of the water in which they swim [5]. These micro-organisms are essentially opportunistic pathogens which invade the tissue of a fish host rendering them susceptible to infection by stress or other disease processes. The most significant group of micro- organisms in this respect is the motile aero monads [6].

A few bacterial species, however, appear to be obligatory parasites of fish. Although they may survive for varying lengths of time in the aquatic environment, they appear to be unable to multiply to any significant extent outside the host. Even the diseases caused by these primary pathogens, however, are almost invariably stress mediated. In general, study of the bacterial diseases of fish is rendered difficult by the current lack of adequate understanding of the interactions taking place between their hosts and the aquatic ecosystem [7]. Some bacterial pathogens such as *Vibrio anguillarum* or *Aeromonas salmonicida* are robust primary pathogens requiring little from their environment or host to facilitates frank infection, others are less able to induce infection and only produce clinical disease in fish that are compromised by their internal or external milieu.

Now there is a wider appreciation of the need to study bacterial diseases in the context of host and environment and such conditions are almost invariably stress related, the exact changes which trigger susceptibility to invasion are not known. Probably they relate to the suppression of the mainly non-specific de-fences such as reticulo-endothelial system or to alterations to the integrity or physiology of the mucoid surfaces [8]. Irrespective of the nature of these mechanisms for enhanced susceptibility, if the fish's resistance is reduced, there will invariably be micro-organisms available to take advantage.

The parasitic infection in turn disturbs the metabolic pathways [9]. Organs and tissue respond to stimuli, either physiological or pathological in various ways, many of which can be identified and studied by histology. These morphological changes can provide the clues necessary to establish a diagnosis. Hence histology is an important tool for diagnosis. With this background the current study was planned to study the structural and histological alterations in gills of *Channa striatus* that are affected with EUS, a disease of economic implications in aquaculture.

2. Material and Methods

Murrels are also known as 'Snake-heads' owing to their shape and appearance of the head which resembles that of a snake i.e. the *C. striatus*. The fish (Size 20 ± 2.4 cm in length 130±35.7 gms in weight) were collected in the Kolleru Lake area of Coastal Andhra Pradesh, designated wetland of international importance since 2002 under the international Ramsar Convention. The sampling was done from October, 2015 to March, 2016, a time during which incidence of EUS was observed to be higher. The fishes were brought to laboratory in live condition, acclimatized to laboratory conditions by growing the fish in freshwater tubs for a couple of days. The acclimmatized fishes were classified with gross observation of lesions as healthy and diseased fish and latter was further classified as mildly infected and severely infected fish. The lesions in infected fish include ulcerations and hemorrhages in fins, along the sides of the body and snout. Skin, gill and fins were examined for lesions and recorded. At the end of the acclimatization the fish were randomly selected for Histopathological studies. The gill tissue was isolated from control and experimental fish and rinsed in Physiological saline solution (0.85% NaCl). Post rinsing the tissue was fixed in aqueous Bouin's solution for 48 h, processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Gills alone were processed by double embedding technique. Sections were cut at 6µ thickness, stained with Ehrlich Hematoxylin/Eosin (dissolved in 70% alcohol) and were mounted in canada balsam [10]. The photographs at 10x magnification were taken with Trinocular microscope with CCD & Monitor (Olympus CX21) Hyderabad, India.

3. Results and Discussion

The gills are important organs for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion [11]. Structurally, the gill arch is a curved bony structure from which radiate double rows of paired primary lamellae or filaments. Each of these primary lamellae was flat leaf like in structure and has a series of secondary lamellae located perpendicular to the primary lamellae with the central supporting axis. The gill arch is covered by typical Teleost epidermal tissue but at the origin of the primary lamellae the epidermis is much thicker and usually contains numerous mucous cells. Below this epidermis there is usually an assay of lymphoid tissue. The primary lamella is covered by a mucoid epidermis which may have salt secreting chloride cells. Gill histology of control fish revealed the intact nature of both primary and secondary gill lamellae (Fig -1).

EUS infected fish had marked pathological changes in its gills. In the mildly infected fish the changes include the bulging of tips of primary gill filaments. There was degeneration of epithelial lining, degeneration in the secondary lamellar of gill was observed. There was fusion of secondary lamellae with irregular lamellar spaces. Structural alterations such as the loss of original contours of secondary gill filaments, epithelial proliferation, lamellar fusion and necrosis were observed (Fig -2).

In severely infected *C. striatus* the damage to their gill tissue was observed to be severe. Shortened and clubbing of ends of the secondary gill lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary lamellae. Hypertrophy and hyperplasia of nuclei were also seen. Apart from all these changes degeneration of secondary and primary lamellae occurred. Dilation and congestion in blood vessels of gill filaments and atrophy of secondary lamellae also observed (Fig- 3). It is possible that the damage of the gills could be a direct result of the salts, heavy metals, pesticides, sewage and fertilizers, which are conveyed to the water [12]. They are directly exposed to poisons and other harm full substances occurring in the external environment, which often cause pathological changes in fish [13].

The gills are among the most vulnerable structure of the Teleost fish because of their external location and intimate contact with the water. So, they are liable to be damaged by any irritant materials whether dissolved or suspended in the water [14]. The substantial surface area of the gills in fish serves as an interface between the environment and blood, mostly for the continuous diffusion of oxygen and the maintenance of acid-base and ion balance [15, 16, 17]. Consequently the EUS infection to the fishes causes alterations in the vital functions and alteration in the morphological structure of the organs.

The diseased fishes will open their gill coverings more widely than the normal ones during respiration and also breathing frequency will become increased. The gill sheets are pale, while sometimes small red spots may show, owing to inflammation. In some cases, the gill sheets are swollen and extended outside gill coverings. Pale gills are always a sign of disease in living fish, the gills of healthy fishes will have a reddish colour, unless they are pigmented in some other species where the colour is dark. In judging the general health of a fish, a simple test may be made in which the eyes and gills play an important role. The substantial surface area of the gills in fish serves as an interface between the environment and blood, mostly for the continuous diffusion of oxygen and the maintenance of acid –base and ion balance [15, 16]. Gills participate in many functions in fish such as respiration, excretion and remain in close contact with external environment particularly sensitive to changes in the quality of the water [18, 19].

It was observed in Bangladesh that fishes which suffered from various diseases such as EUS, fungal, parasitic, bacterial infections in most cases had haemorrhages, septicemia, lesions in the gill [20,21]. Indian major carps are highly susceptible to diseases in comparison to Chinese and European carps [22]. Ahmed *et al*., 1998 [23] found that

hypertrophied gill lamellae with loss of secondary gill lamellae were evidenced in *Cirrhinus cirrhosus* in private ponds in Bangladesh. Akter *et al*., 2009 [24] observed that structure of gill of *Anabas testudineus* changes such as hypertrophy; clubbing and few lamellar missing were recorded. Similar findings were also recorded by Hoque, 1995 [25] with the gill lamellae of *C. cirrhosus*. The epithelium that covers the gill filaments and lamellae provides a distinct boundary between a fish's external environment and extracellular fluids and also plays a critical role in the physiological function of the fish gill. The gill epithelium of the fish is the major site of gas exchange, acid – base balance, ionic regulation and excretion of nitrogenous waste [26].

Hassan *et al*., 2010 [27] reported that *Clarias gariepinus*, the altered skin and gill were confirmed by the detection and identification of pathogenic mixed bacteria such as Actinobacteria Enterobacter; *E. coli; Citrobacter*; *Aeromonas* and *Staphylococcus*. Ahmed and Shoreit, 2001 reported *Aeromonas* and *Pseudomonas* sp [28] in EUS infected fish; Koteswarrao and Benarjee, 2015 [29] reported that *Channa punctatus* infected with fungal *Aspergillus fumigatus* and *A. niger* exhibited EUS characters. The histological alterations found in the gills were dilation of the marginal channel, hyperplasia of the epithelial cells and lifting of the lamellar epithelium. In severe cases, necrotic changes with degraded lamellar epithelium were observed. The fungal hyphae encapsulated by fused multiple layers of secondary lamellae too were found [30]. Some severe lesions found in the gill were lamellar haemorrhages with rupture of the lamellar epithelium [31]. The gills of infected *Labeo rohita* fingerlings with *Vibrio cholerae* showed proliferation of filamentary epithelium, lamellar fusion, loss of secondary lamellae, swelling of inter lamellae and excessive secretion of mucus on the surface of filaments. The cellular changes observed in the gills in terms of epithelial proliferation, separation of epithelial layer for supportive tissues and necrosis can adversely affect the gas exchange and ionic regulation [32]. The results of the present study clearly indicated occurrence of EUS in *C. striatus* with fungal aetiology and mycotic granulomatosis response against the invading bacteria.

Histopathology of the Gill of *Channa striatus*

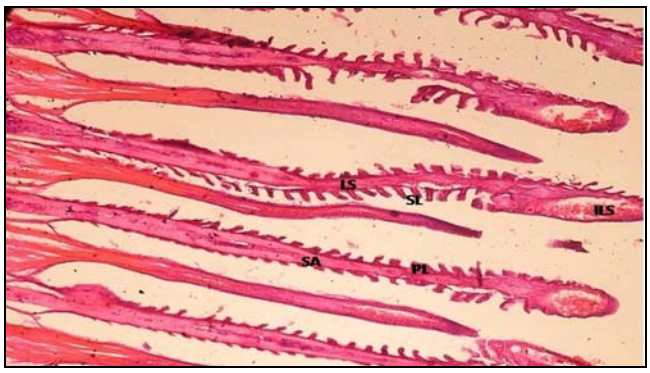

Fig 1: Control Gill of *Channa striatus*

PL – Primary lamellae; SL – Secondary lamellae; LS – Lamellar space; ILS – Inter lamellar space; SA – Supporting axis

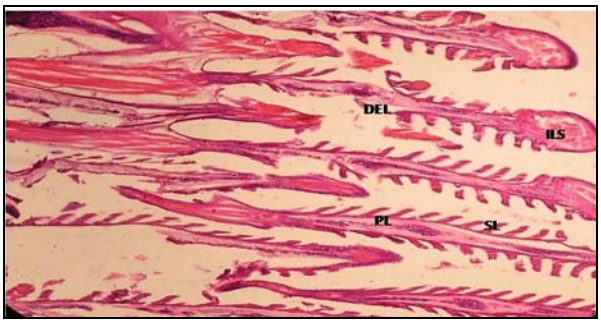

Fig 2: Gill of Mildly infected *Channa striatus* with EUS

SL – Secondary lamellae; DEL – Degeneration of epithelial lining; ILS – Inter lamellar space;
PL – Primary lamellae

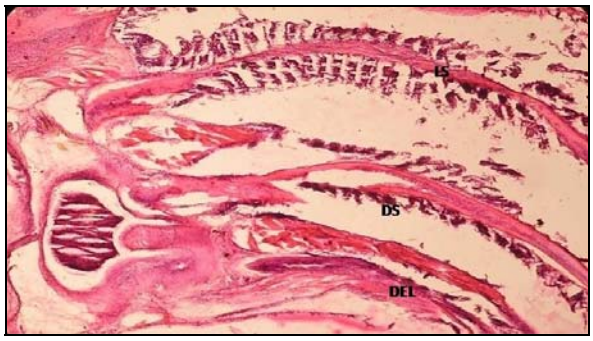

Fig 3: Gill of Severely infected *Channa striatus* with EUS

LS – Lamellar space; DEL – Degeneration of epithelial lining; DS – Degenerated secondary lamellae

4. Conclusion

It can be concluded from our study that EUS causes degenerative changes in the gill of *C. striatus* thereby reducing their viability and growth. The extent of these changes depends on the severity of the infection which in turn depends on the other predisposing factors like host immunity and environmental factors. These histopathological changes in gills can alter various physiological parameters of fish resulting in abnormal metabolism.

5. Conflict of Interest Statement

The authors have no conflict of interest with regard to the contents of this manuscript.

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