

Microbial Flora from the Epizootic Ulcerative Syndrome (EUS) Infected Murrel *Channa striatus* (Bloch, 1797) in Tirunelveli Region

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Abstract: The microbial flora of the liver, gills, intestine, and muscle of epizootic ulcerative syndrome (EUS) infected murrel *Channa striatus* in Tirunelveli, Tamilnadu was estimated quantitatively and qualitatively, and the isolates were identified at genus and species level. Total viable microbial count was measured as highest range in gills ($5.9 \pm 0.5 \times 10^7$ CFU (colony forming units) g^{-1}) and lowest range in intestine ($8.7 \pm 1.8 \times 10^4$ CFU g^{-1}). In total 17 bacterial and mycotic species were isolated and identified and *Aeromonas hydrophila*, *Enterobacter* sp., *Vibrio* sp., *Pseudomonas* sp., *Escherichia coli*, *Aphanomyces invadans*, and *Aspergillus* sp. were the most predominant isolated and identified species in all samples. The purpose of this study is to isolate and identify the microflora of EUS infected *C. striatus*.

Key Words: *Channa striatus*, EUS, microbial flora, Tirunelveli

Murrels, commonly called snakeheads belonging to the family Channidae (Ophiocephalidae), constitute the most common and dominant group of air breathing freshwater fishes and are highly regarded as food fish in the South and Southeast Asian countries (1). It has long been commercially cultured in Thailand, Taiwan, and the Philippines. There are several species of murrels belonging to the genus *Channa* (syn. *Ophiocephalus*), but only one species, namely *Channa Striatus* also called striped murrel, enjoys a good deal of popularity as food fish in many parts of India (2). Besides the high quality of their flesh in terms of taste and texture, they also have good market value due to the low fat, fewer intramuscular spines, and medicinal qualities (3). Over the past 2 decades, epizootic ulcerative syndrome (EUS) had a serious impact on tropical fisheries resulting in heavy economic loss (4). It is one of the most destructive diseases amongst fresh and brackish water fish in the Asian Pacific region and it is very common in both northern and southern India and has spread through rivers, reservoirs and paddy fields to neighboring states, causing considerable loss to fish farmers (5). In the initial stages, the disease is marked by little red spots on the skin surface, which progress in size until eventually, a

circular to oval deep haemorrhagic ulcer exposing the skeletal musculature is visible. A diverse group of biotic agents such as viruses, bacteria, and cutaneous ectoparasites may initiate skin lesions, which are subsequently colonized by *Aphanomyces invadans* and ultimately lead to EUS (6). Different pathogenic organisms, including bacteria (7,8), fungus (9) and virus (10), have been reported to be isolated from naturally infected fish. Roberts et al. (11) reported that in natural outbreaks more than 100 fish species, especially air breathing fishes, had been affected by EUS.

The present attempt is the first study undertaken to assess quantitatively and qualitatively the bacterial and mycotic species from the EUS infected murrel *C. striatus* collected in and around Tirunelveli region during 2006.

A total of 115 infected striped murrels (*C. striatus*) of average mean length (15 ± 2 cm) and average weight (120 ± 3.5 g) were collected from Tamiraparani river fed systems, Tirunelveli [$8.44^\circ N$, $77.44^\circ E$], Tamilnadu, India in the month of February 2006 (Figure). The water temperature (28 ± 1.5 °C), dissolved oxygen (5.8 mg/L) and pH (7.1-7.4) were observed during the collection period. They were transported to the Centre for

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Aquaculture Research and Extension (CARE) Aquafarm in live condition with oxygenated water in plastic bags (5 l) and they were acclimatized in cement tanks (3 m × 12 m × 1 m) for 24 h. All the infected individuals showed external symptoms like unresponsiveness, wound infection, irregular pattern, superficial lesions, swelling discoloration, and deep ulcer hemorrhages. One gram of each samples of muscle, liver, gills, and intestine of the infected fish were taken and processed individually. Whole subcutaneous tissues beneath the margin of the ulcers were scraped thoroughly and used for mycological studies. The samples were pooled and inoculated onto tryptic soy agar (Himedia, Mumbai) and the inoculated plates were incubated at 28 °C for 24-48 h. After the incubation, the colony forming units (CFU) were counted under a Quebec dark field colony counter with a guide plate ruled in square centimeters. Plates containing 30 - 300 colonies were used to calculate microbial population results, recorded as CFU per unit of a sample. The isolates were identified from each sample. After

incubation, the microbial colonies were divided into different types according to colonial characteristic morphology in order to determine the present composition. In order to obtain pure culture, 3-5 representatives of each colony type were streaked and restreaked on fresh media. The genus and species of selected bacterial isolates were identified by various biochemical tests using the criteria provided in Bergey's manual of systemic bacteriological classification (12). Fungal species were identified according to their morphology (Nikon-Eclipse E400 microscope, Germany) using potato dextrose agar and Czapek Dox agar (Himedia, Mumbai) (13). Data are presented as mean ± standard deviation (SD).

The results of the quantitative estimation of microbial count in the liver, gills, intestine, and muscle of diseased *C. striatus* are given in the Table 1. The highest microbial load was observed as $5.9 \pm 0.5 \times 10^7$ CFU g⁻¹ in gills. The lowest load found in intestine and muscle was $8.7 \pm 1.8 \times 10^4$ CFU g⁻¹ and $9.3 \pm 2.4 \times 10^4$ CFU g⁻¹, respectively. The percentage distribution of bacterial and mycotic isolates is shown in Table 2. Fourteen bacterial species and 3 fungal species were isolated and identified. Among the 17 isolates, dominant bacterial and fungal species were *Aeromonas hydrophila*, *Flavobacterium* sp., *Aspergillus flavus*, and *Aphanomyces invadans*. The following was the most frequently isolated bacterial and mycotic flora with a prevalence of greater than 10%: *Aeromonas hydrophila*, *Aeromonas* sp., *Flavobacterium* sp., *Vibrio vulnificus*, *Staphylococcus* sp., *Yersinia enterocolitica*, *Shigella* sp., *A. salmonicida*, *Aspergillus flavus*, *Aspergillus* sp., and *Aphanomyces invadans*. From 25 microbial colonies in gill samples, 11 colonies of *Aeromonas hydrophila* were identified predominantly.

The presence of high bacterial load in the gills and intestine of hybrid tilapia was reported as $7.1 \pm 0.7 \times$



Figure. Collection site-Tirunelveli.

Table 1. Microbial load in liver, gill, intestine, and muscle of diseased *Channa striatus* (Values are mean ± SD).

Sample (n = 115)	Colony Forming Unit g ⁻¹ (CFU g ⁻¹)
Liver	$7.3 \pm 1.1 \times 10^6$
Gill	$5.9 \pm 0.5 \times 10^7$
Intestine	$8.7 \pm 1.8 \times 10^4$
Muscle	$9.3 \pm 2.1 \times 10^4$

Table 2. Result of bacterial and mycotic isolates from different organs of infected *Channa striatus*. (no. - number of isolates; % - percentage of each isolated organisms).

Organism	Liver		Gills		Intestine		Muscle	
	no.	%	no.	%	no.	%	no.	%
<i>Aeromonas hydrophila</i>	9	34.61	11	35.48	2	20	3	18.75
<i>Aeromonas</i> sp.	8	30.76	3	9.67	1	10	-	-
<i>Flavobacterium</i> sp.	5	19.23	4	12.90	-	-	-	-
<i>Bacillus mycoides</i>	1	3.84	2	6.45	-	-	-	-
<i>Bacillus</i> sp.	-	-	2	6.45	-	-	-	-
<i>Pseudomonas fluorescens</i>	-	-	1	3.22	-	-	-	-
<i>Vibrio vulnificus</i>	-	-	2	6.45	-	-	3	18.75
<i>Staphylococcus</i> sp.	-	-	-	-	-	-	1	6.25
<i>Yersinia enterocolitica</i>	-	-	-	-	1	10	-	-
<i>Shigella</i> sp.	-	-	-	-	2	20	-	-
<i>Aeromonas salmonicida</i>	-	-	-	-	3	30	-	-
<i>Vibrio alginolyticus</i>	-	-	-	-	1	10	-	-
<i>Proteus rettgeri</i>	-	-	-	-	-	-	1	6.25
<i>Enterobacter agglomerans</i>	-	-	3	9.67	-	-	-	-
<i>Aspergillus flavus</i>	3	11.53	2	6.45	-	-	3	18.75
<i>Aspergillus</i> sp.	-	-	1	3.22	-	-	2	12.50
<i>Aphanomyces invadans</i>	-	-	-	-	-	-	3	18.75

$10^5 - 8.7 \pm 1.1 \times 10^6$ CFU g^{-1} and $3.4 \pm 1.8 \times 10^5 - 5.8 \pm 0.4 \times 10^7$ CFU g^{-1} , respectively (14). In the present study the mean bacterial load was observed to be more dominant than fungi (in gills and liver $5.9 \pm 0.5 \times 10^7$ CFU g^{-1} and $7.3 \pm 1.1 \times 10^6$ CFU g^{-1} , respectively). In Bangladesh, *C. punctatus* and *Labeo rohita* were affected by various ulcer-type diseases, such as epizootic ulcerative syndrome, bacterial hemorrhagic septicemia, tail rot, fin & gill rot, fungal disease, and parasite disease (15). In the present study, *C. striatus* was affected by EUS in Tirunelveli district. *A. hydrophila* is one of the most important freshwater fish pathogen isolated from EUS affected fish (8,16). Bacterial flora of some freshwater fishes in tropical water showed that *Aeromonas* sp. was the most predominant microorganism isolated from the skin, gills and intestine of the fish (17,18). Rich data are also available regarding the pathogenic mechanism and virulence of *A. hydrophila*, which caused mass mortality in cultured Japanese catfish larvae (19). The outbreak of a disease, which had more than 75% mortality among Indian major carps, was found mainly due to *A. hydrophila* (20). Similarly, *A. hydrophila* was also identified from freshwater fish *C. striatus* in the present study. Lio-Po et al. (21) reported that several species of

bacteria and fungi were found to be associated with EUS affected snakehead *C. striatus* and that 89% of the total isolates were *A. hydrophila*. Furthermore, 25 bacterial and fungal species were isolated and identified in freshwater carp at Himachal Pradesh, India (22) and 15 isolates of bacteria in hybrid tilapia from Saudi Arabia (14). In the present study, a total of 17 bacterial and mycotic species were isolated and identified in Tirunelveli district, with most of the isolates from muscle and gills. The gill disease in Hungary during winter and spring in sheath fish and silver carp was reported (23). *Aphanomyces invadans*, a highly invasive, specific, slow growing fungus, cause epizootic ulcerative syndrome (24). Mycobiotic agents, *Aspergillus flavus* and *Aspergillus fumigates*, were the main fungi isolated from the Nigerian freshwater fish culture (25). In the present investigation, mycotic species, such as *Aphanomyces invadans*, *Aspergillus flavus*, and *Aspergillus* sp. were isolated from the infected fish samples.

In conclusion, to identify the causative agents of murrel disease, further molecular level research on this subject to know the disease mechanisms and the methods of prophylaxis and treatment is required.

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