Haematological changes in South Indian fresh water murrel, *Channa punctatus* have both EUS and *A. hydrophila* infection

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Abstract The mixed infection in fishes is a common feature and in this Pathogens like Bacteria, Fungi and Protozoan's are found together to cause ill health to the fishes known as Epizootic Ulcerative Syndrome (EUS). In this syndrome the low temperature and aquatic pollution aggravates the infection in fishes. In the present study the fresh water edible fish, *Channa punctatus* (Murrel) was found infected with the bacteria namely, *Aeromonas hydrophila*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella salmonicida*. The fungi, *Aphanomyces invadans* was also found. The major objective of this study is to understand the microbial intensity in various organs of fish and hematological variations in both control and EUS infected fish. The highest microbial load of $8.2 \pm 0.12 \times 10^7$ cfu g⁻¹ was observed in the skin where as the lowest load of $3.2 \pm 0.8 \times 10^3$ cfu g⁻¹ was found in the pancreas. From the microbial diagnosis, *A. hydrophila* has been isolated from different organs indicating its predominant presence. In comparison with the control the haematological indices like WBC, MCV, Lymphocytes, Eosinophils and Basophils were found increased in the infected by (17 %), (16 %), (5 %), (58 %) and (27 %) respectively. The variations in microbial load found in fish in the study can be attributed to feeding habits, behavior, fish adaptations among the different fish species. These conditions are being caused by the presence of environmental pollution and are high in heavy native fishes. Thus the results showed that low of Haemoglobin value Packed Cell Volume and Red Blood Corpuscles indicated the presence of anemic conditions in the EUS infected fish found in Warangal.

Keywords *Channa punctatus* · Bacteria · Fungi · Environment factors · Epizootic Ulcerative Syndrome (EUS)

Introduction

Murrel fishes belong to the family Channidae which consists of about 28–30 species. There are about 8–10 species are found in India. They are *C. straitus*, *C. marulius*, *C. punctatus*, *C. micropeltes*, *C. guchua* and *C. diplogramma* which are highly prized fish and got rich food value. However the Ornamental fish species are *C. bleheri*, *C. Orientalis*, *C. aurantimaculate*, *C. Asiatics* and *C. stewarti* (Haniffa et al. 2004). Murrels are air breathing fresh water fishes and are the highly prized food fish in South-East Asian countries due to their taste, flavour, fewer intra muscular spines, salutatory and reproductive prosperities. Murrels are very often affected by Epizootic Ulcerative Syndrome (EUS) and it causes huge loss to the capture and culture fisheries sector. The principal infectious agent of this infestation is a fungal species, *Aphanomyces invadans* (Mohan and Shankar 1995). Whereas the opportunistic bacterial infectious agent i.e. *Aeromonas hydrophila* is also found with this fungi (Karunasagar et al. 1995). These pathogens seize the blood circulation and resulting edema of the abdominal cavity (Pal and Pradhan 1990). The epithelial layer of the skin, gills and alimentary tract, confer potential sites for these pathogens (Gobinath and Ravichandran 2011). The fresh water fishes belonging to different taxonomic groups are adapted to different ecological conditions. The haematological characteristics are one of the important tools that can be used to understand as an effective and sensitive index to monitor physiological

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and pathological changes in fishes. Changes in the Haematological parameters depend the aquatic biotope, fish species, age, sexual maturity and health status (Patriche et al. 2011; Radu et al. 2009). Various blood parameters in fish have been estimated by different investigators in order to understand the fish physiology and pathology (Vázquez and Guerrero 2007; Satheeshkumar et al. 2012; Fazio et al. 2013). Haematological and Biochemical studies helps in understanding the relationship of blood characteristics to the habitat and adoptability of the fish to the environment. The haematological parameters of the fish, *Channa punctatus* such as RBC, WBC, HB, PCV, MCV, MCH, MCHC and DLC etc. are thus shown to be influenced by many factors include environmental factors (Pandey 1977). Therefore the Aqua culturists have studied these varying aspects of haematological and biochemical changes in fish at different environmental conditions. The present study deals with the comparisons of blood parameters and estimation of microbial flora cultures in various organs of *Channa punctatus* infected with EUS (Figs. 5, 6, 7, 8).

Materials and methods

Fresh water *Channa punctatus* control and infected live fishes (weight 120 ± 2 g) were collected from the landing sites of Dharmasagar, Hasanparthy and Bhandham lakes of Warangal district of Telangana. During the period (September 2013–March 2014), the infected *Channa punctatus* which exhibited pathological characteristic features of Epizootic Ulcerative Symptoms externally on skin, lower jaw, tail and fins. Figure 1 and control fish, *Channa punctatus* were also used in this study (Figs. 2, 3 4, 5).

Isolation and identification of bacterial pathogens from fish organs

Collection of fish material from internal organs and external skin examined fishes were streaked on to Nutrient Agar (NA) Trypticase Soy Agar (NA) plates then incubated

Fig. 1 Control *Channa punctatus*

Fig. 2 Infected *Channa punctatus* with *Aeromonas hydrophila*: (external hemorrhage and tail rot)

Fig. 3 Skin ulceration

at 24–48 h. the growing colonies were picked up in pure form and reincubated into Trypticase Soy Agar for further identification. Identification of all isolates was done by cultural, morphological and biochemical characters according to Quinn et al. (2002), Austin and Austin (2007) and through using API-20E (Biomérieux) for gram-negative fish pathogen (Figs. 6, 7, 8; Table 1).

Collection blood

Few drops of blood was drawn from the caudal vein by introducing disposable sterile syringe (2.5 ml) and transferred to miniplast 0.5 ml tube containing EDTA (1.26 mg/0.6 ml) as an anticoagulant for blood cell studies. The collected blood samples were immediately subjected to haematological analysis. Clinical examinations of the fish were done according to Austin and Austin (2007). Haematological parameters and the haematological indices (Red Blood Cell count White Blood Cell count, Differential Leukocyte Count, Haemoglobin%, PCV, MCV, MCH, MCHC) were calculated by using standard formulae of Campbell (2004). To estimate the haematological parameters the blood samples were collected during the morning hours to avoid diurnal variations (Acharya and Mohanty 2014). The blood was diluted with appropriate diluting fluids for RBC and WBC counts and was determined using

Fig. 4 Dermal ulceration of lower jaw

Fig. 5 *Aeromonas hydrophila* culture on Nutrient Agar (NA) and (SA)

Fig. 6 *Staphylococcus aureus* culture on Nutrient Agar (NA) and (SA)

Fig. 7 *Pseudomonas aeruginosa* culture on Nutrient Agar (NA) and (SA)

Fig. 8 *Salmonella salmonicida* culture on Nutrient Agar (NA) and (SA)

Table 1

Blood parameters	Control fish	Infected fish	Percentage change (%)	P values
HB (g/dl)	10.1 ± 0.33	9.1 ± 0.29	−90.9	0.0373
Haematocrit (PCV) %	32.7 ± 0.68	28.3 ± 0.47	−71.7	0.0001
MCV (M g)	56.7 ± 0.94	65.7 ± 1.32	−34.3	0.0001
MCH (M g)	21.4 ± 0.61	18.9 ± 0.31	−81.1	0.0015
MCHC (M g)	36.539 ± 0.925	33.159 ± 0.174	−66.841	0.0021
RBCs (10 ⁶ /ml)	4.2 ± 0.27	1.89 ± 0.13	−98.11	0.0001
WBCs (10 ³ /ml)	33,505 ± 674.13	39,000 ± 756.450	38,900	0.0001
Lymphocytes %	58.2 ± 0.341	61 ± 0.614	−39	0.0009
Neutrophils %	34.8 ± 0.64	29.3 ± 1.67	−70.7	0.0059
Monocytes %	5.8 ± 0.37	4.4 ± 0.74	−95.6	0.0057
Eosinophils %	2.6 ± 0.37	4.1 ± 0.38	−95.9	0.0124
Basophils %	2.2 ± 0.28	2.8 ± 0.38	−97.2	NS

improved Neubauer haemocytometer and calculated as per (Blaxhall and Daisley 1973). Replicated counts were made for each blood sample to minimize the error. The Hemoglobin content was determined by using hemoglobin test kit (DIAGNOVA, Ranbaxy, India). The blood film was prepared and stained with Giemsa stain for morphology, micrometry and Differential Count of Leucocytes. Haematocrit (Hct) was determined by the Microhaematocrit method (Snieszko 1960). Mean Corpuscular Volume (MCV) was calculated according to Feldman et al. (2000). Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Haemoglobin concentration (MCHC) were calculated according to Stoskopf (1993).

Results and discussion

The aetiology of EUS in fresh water fishes of *Channa punctatus* a year of survey was carried out and it was found that the bottom dwelling fishes. The *Channa punctatus* were mostly affected by fishermen to the market from three lakes, Dharmasagar, Hasanparthy and Bhandham. Then it was decided to study the haematological parameters and

estimation of microbial flora in various organs of these three lakes. The infected *Channa punctatus* fish shows diseased symptomatic characters. The naturally infected *C. punctatus* were skin ulcers or sores on the dorso—ventral surface of the body, edema of the fins and the vent, and anorexia. This type of ulcers said to have characteristic “punched out” appearance, i.e. white area with reddish centre turning into complete red area which later merged as the disease progress it further resulted in external haemorrhages, distended abdomens and protruding eyes leading to death of the fish due to severity of the disease. The present study was aimed to estimation of the different bacterial loads on the skin, gill, liver and pancreas of *C. punctatus* EUS infected fish. Isolated bacteria from the ulcerated area and in the external and internal organs such as skin, gills, liver and pancreas. Isolation of bacteria were found in the infected fish had *A. hydrophila*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella salmonicida*. These bacteria were determined in the Dharmasagar, Hasanparthy and Bhandham lakes. The highest microbial load of $8.2 \pm 0.12 \times 10^7$ cfu g^{−1} was observed in Skin and in the Gills $6.4 \pm 0.4 \times 10^5$ - cfu g^{−1}. Whereas the Pancreas was recorded the lowest

Table 2 The total heterotrophic count in *C. punctatus* diseased of different organs

S. no.	Sample of fish organ	Colony forming unit (cfu g ⁻¹)
1	Skin	$8.2 \pm 0.12 \times 10^7$
2	Gill	$6.4 \pm 0.4 \times 10^5$
3	Liver	$4.5 \pm 0.5 \times 10^3$
4	Pancreas	$3.2 \pm 0.8 \times 10^3$

Table 3 The distribution of bacterial pathogens in infected *Channa punctatus*

S. no.	Isolation of bacterial pathogens	Distriubiton of pathogens (%)							Total (%)
1	<i>Aeromonas hydrophila</i>	40	5.0	1.0	2.5	1.0	3.0	52.5	
2	<i>Staphylococcus aureus</i>	3.5	1.5	1.0	1.0	0.5	1.0	8.5	
3	<i>Pseudomonas aeruginosa</i>	8.5	2.5	1.0	1.5	1.0	0.5	15.0	
4	<i>Salmonella salmonicida</i>	1.5	0.2	0.5	1.0	–	0.3	2.6	

value ($3.2 \pm 0.8 \times 10^3$ cfu g⁻¹) (Table 2). While in the isolated bacteria were *A. hydrophila* was found as the dominant one (Table 3). Dominannce presence of *A. hydrophila* in the EUS affected *C. striatus* was reported by Thampuran et al. (1995). According to Das (1997). EUS affected fishes all over the world recorded a wide range of pathogenic bacterium *A. hydrophila* as the most prevalent where as *Staphylococcus aureus* sp., *Pseudomonas* sp. and *Salmonella* sp. The majority of bacterial pathogens are natural inhabitants and normal commensalism of the aquatic system, but are opportunistic pathogens commonly occurring secondary to extrinsic stressors including crowding, mechanical injury due to rough handling poor water quality insufficient aeration, pH, water hardness, temperature and NH₃ poisoning (Kavitha et al. 2014). And also the present investigation deals with the comparison of blood parameters with special reference to the microbial flora of fresh water fish of *Channa punctatus* infected and control fish.

In the EUS affected murels captured from the wild, different pathogenic organisms including Fungi, *A. invadans*, (Chinabut 1995), bacteria *A. hydrophila*, *Vibrio* sp., (Pal and Pradhan 1990) and Virus (Frerichs 1995) have been detected. Among the fish potential pathogens were identified, *Aeromonas* and *pseudomonas* species pose the most significant threats to murrel fishes. *A. hydrophila* causes a variety of non specific clinical signs including external haemorrhages of the skin and fins, dermal ulcerations, abdominal destinations, exophthalmia and septicemia. Other bacterial pathogens include *A. salmonicida*, *Pseudomonas* sp and *Salmonella* sp., *Flavobacterium columnare*, *Mycobacterium* sp., such as *M. marinum*, *M. fortuitum* and *M. piscium* common infectious disease of

ornamental fish. Similarly, Manohar (2005) has also reported the dominance of *A. hydrophila* in the infected *C. carpio*. Motile group of Aeromonads have been complete with lesions on dorso—ventral surfaces ((Llobrera and Gacutan 1987; Lio-Po et al. 1992).

Statistical analysis

Haematological parameters of fresh water *Channa punctatus* control and bacterial infected fish were statistically evaluated with the students 't' test and the results are presented as Mean and Standard Deviations (SD).

The predominance of *A. hydrophila* in EUS affected fish has also been reported previously by Kumar et al. (1990) in India; Tonguthai (1985). In Thailand, Wong and Leong (1987) in Malaysia, Dana (1987) in Indonesia; Roberts (1997) in Mayanamar and Balasurya (1987) in Srilanka. Lio-Po et al. (1992) reported that bacterial and fungal pathogens were noted to be related with EUS affected Snake head *C. striatus* and 80 % of the total isolates were *A. hydrophila*. Smith et al. (2012) categorized the different groups of bacterial pathogens in the aquarium water where gold fish and Chinese algae eaters thrived using molecular appearances and identified the presence of 30 phyla of pathogenic microbes with Proteobacteria (52 %), Bacteriodes (18 %) and planctomycetes (60 %), representing the top three phyla. In this infected *Channa punctatus* showed a decreased values of HB (10.1 ± 0.33 to 9.1 ± 0.29 g/dl), PCV (32.7 ± 0.68 to 28.3 ± 0.47), MCH (21.4 ± 0.61 to 18.9 ± 0.31), MCHC (36.539 ± 0.925 to 33.159 ± 0.174), RBC (4.2 ± 0.27 to 1.89 ± 0.13), Neutrophils (34.8 ± 0.64 to 29.3 ± 1.67 %), Monocytes (5.8 ± 0.37 to 4.4 ± 0.74 %) which were decreased significantly ($P < 0.0001$) than that of control fish. WBC ($33,505 \pm 674.13$ to 39000 ± 756.450), MCV (56.7 ± 0.94 to 65.7 ± 1.32), Lymphocytes (58.2 ± 0.341 to 61 ± 0.614 %), Eosinophils (2.6 ± 0.37 to 4.1 ± 0.38 %) this values of EUS infected *Channa punctatus* fish showed a significant increase when to that of control fish. The basophils exhibited an increased in their count in the infected 2.8 ± 0.38 % while in the control fish the value has to 2.2 ± 0.28 %. These values are insignificant. The study of the RBCs in combination with poor HB mobilization from the Spleen to other hemopoietic organs resulted in decreased HB and RBC levels due to hypochromic microcytic anemia of the fish infected with *A. hydrophila* (Scott and Rogers 1981). The results showing that the level of HB, values of PCV, MCH, MCHC and the number of RBCs were significantly decreased in *Channa striatus*, fish injected with *A. hydrophila* when compared to that of control fish Haniffa et al. (2011) Similarly, decreased levels of RBCs, and PCV were noticed in Red Salmon, Rain bow Trout, Carp, Nile Tilapia infected by

Vibrio anguillarum Harbell et al. (1979), *A. Sobria* and *A. Cavia* Řehulka (2002), *A. hydrophila* Hari Krishnan et al. (2003), *Streptococcus iniae* Chen et al. (2004) respectively. The pearl spot fish *E. Suratensis* is when affected by EUS becomes anemic and in turn results in the significant reduction of RBC, HB, and PCV levels (Pathiratne and Rajapakshe 1998). The MCHC as a best indicator of RBC swelling/decrease in HB synthesis (Wepener et al. 1992). The increased level of lymphocytes obtained in our study is supported by Morgan et al. (1997) in infected brown trout and rainbow trout.

Conclusion

The predominant presence of *A. hydrophila*, *P. aeruginosa*, *S. aureus* and *S. salmonicida* were naturally infected in *Channa punctatus* insisted its primary role in the ulcer disease. However further diagnostics regarding the fungal pathology is required. The present study is significant that is the first report on the occurrence of Epizootic Ulcerative Syndrome disease in the fresh water Teleost fish of *Channa punctatus*.

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