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Incidence of epizootic ulcerative syndrome (EUS) in freshwater fishes in the endemic area of Punjab, Pakistan

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Abstract
Incidence of Epizootic Ulcerative Syndrome (EUS) has been recorded for the first time in freshwater fishes in the endemic area of Punjab, Pakistan. Survey of private fish farms, hatchery and natural water bodies was conducted in a radius of 14 Km from around river Ravi near Lahore (Punjab Province) Pakistan. Out Of 1628 fishes belonging to 18 genera, 517 fishes of 10 genera were found affected with EUS. The incidence of EUS in culturable fishes was higher in *Cirrhina mrigala* (15.4%) moderate in *Catla catla* (13.3%) and lower in *Labeo rohita* (5.0%). Exotic fish, Chinese carp *Ctenoparyngodon idella* and *Hypophthalmicthys molitrix* were not affected with EUS. In non-culturable fishes the incidence of EUS was highest in *Channa punctatus* (72.8%) moderate in by *C. straitus* (65.45%) and comparatively lower *Puntius ticto* (43.7%). A slow growing temperature sensitive *Saprolegnia spp.* was isolated from all of EUS infected fish species. *Aeromonas spp. and Pseudomonas spp.* were isolated from the diseased fishes. Ectoparasites *viz. Lernaea, Argulus* and *Trichodina* spp. were also isolated from the skin and gills of infected fish species. The disease was more severe in water having low alkalinity (70 mg/l), hardness (75 mg/l) and low temperature of 10-12 ^{0}C.

Keywords: EUS, Bacteria, Water quality

Introduction

The Epizootic Ulcerative Syndrome (EUS) is a condition characterized by large cutaneous ulcerative lesions that periodically results in death in many species of wild and cultured freshwater fish, often involving a number of pathogens (FAO 1986). The same disease is also called Mycotic Granulomatosis (MG) in Japan (Egusa 1992) and Red Spot Disease (RSD) in Australia (Callinan *et al.* 1995). This disease was first noticed in 1972 in Australia (Chattopadhyay *et al.* 1990) and Southeast-Asia (Rodgers and Burke 1981). Its presence has also been reported in Southeastern United States (Noga and Dykstra 1986). It appears as if EUS is spreading more widely prevalent in Asian Countries. This has been reported from Philippines, (Reantaso 1990), eastern and western India (Das *et al.* 1990), Srilanka (Frenichs 1988), Bangladesh (Roberts *et al.* 1989), Bhutan (Phillips 1989) and Nepal (Shrestha 1990). EUS is believed to have

entered India in 1988 and since then has been causing large-scale mortalities in both freshwater and brackish water fish (Das and Das 1993). Since Pakistan share a long eastern border with India, there was a possibility that disease could have been transmitted depending on aquatic environment contamination.

We in our preliminary survey in the Potohar Region of northern Punjab of Pakistan did not find presence of EUS (Rab *et al.* 1993). Recently some complaints of disease outbreak similar to EUS were received from Punjab (Pakistan). The present investigation in from of a comprehensive survey therefore, was designed to determine the incidence of EUS in different fish species, both in culture system and wild of Punjab, Pakistan

Materials and methods

A survey of water bodies was conducted from April to July'97, in a radius of 14 km around River Ravi near Lahore (Punjab Province), Pakistan. Four private fish farms, one private fish hatchery, 20 natural water bodies in the form of small and large ponds called Dhunds (mainly non drainable) and catches from River Ravi were included in the study.

The study area is located in flooded zone of Pakistan. Out of the total rainfall in these areas, about 70 percent are received during monsoon rainy season (July-September). The maximum temperature ranges from 40-45 ^{0}C in June. In winter, the temperature ranges between 10^{0}C-25^{0}C (November-February) in this area.

A total number of 1628 different fishes representing 18 genera were examined for the presence of EUS according to the previously described signs and symptoms (Touguthai 1985, Roberts *et al.* 1989, Shrestha 1990 and Prasad and Sinha (1990). The fishes were collected with the help of cast nets and from commercial catches of River Ravi.

Parasites were isolated and identified as described by Jaffry (1995). For bacteriological and fungal investigation, fishes were stored in ice and immediately transported to laboratory, as described by Callinan *et al.* (1989). EUS infected fishes (25) were preserved in 10% buffer formalin and sent to Aquatic Animal Health Research Institute (AAHRI) Bangkok, Thailand, for confirmation of EUS through Network of Aquaculture Center in Asia-Pacific (NACA).

Water quality parameters were tested on the spot at all sampling sites, using Digital Titrator (Model 16900-01) HACH water analysis kit and have been presented in Table1.

Table 1. Water quality parameters of different sites in the endemic area of Punjab, Pakistan

	Private fish farms	Natural water bodies	Ravi river
Temperature (°C)	12-27.5	11-27	10-26
pH	8.0-8.5	8.0-8.5	7.5-8.0
DO (mg/l)	7.1-10.6	7.9-10.5	2.5-9
Alkalinity (mg/l)	100	70	121

Acidity (mg/l)	20-70	42-86	42-61
Chloride (mg/l)	3.6-5.0	5.6-6.0	4.2-7.8
CO_2 (mg/l)	23.0-46.0	30.8-38.0	30.0-40.3
Hardness (mg/l)	95-120	75-110	110-230
Ammonia (mg/l)	0.1-0.2	Nil	0.7-1.3
Nitrite (mg/l)	Nil	Nil	Nil

Results

The incidence of EUS in freshwater fishes in the endemic area of Punjab in Pakistan is presented in Table2. The incidence of EUS in non-culturable fishes was highest in *Channa punctatus*(72.72%), moderate in *Channa straitus*(65.45%), and comparatively lower in *Puntius ticto* (43.66%). The incidence of EUS in culturable fishes was higher in *Cirrhinus mrigala* (15.38%), moderate in *Catla catla*(13.33%) and lower in *Labeo rohita*(4.96%). The clinical signs and symptoms related to EUS and exhibited by these different fish species in the endemic area of Pakistan are presented in (Table 2).

Table 2. Incidence of epizootic ulcerative syndrome in freshwater fishes in the endemic area of Punjab, Pakistan

Fish species	Incidence (no. infected/ no. observed)	Signs & symptom*			
		ul	ef	ha	rs
Culturable fishes					
1. *Cirrhina mrigala*	15.4(10/65)	+	+	-	+
2. *Catla catla*	13.4(22/165)	+	-	+	-
3. *Labeo rohita*	5.0(15/302)	+	-	+	-
Non-culturable fishes					
1. *Channa punctatus*	72.8(200/275)	+	+	+	+
2. *C. straitus*	65.5(108/165)	+	+	+	+
3. *Puntius ticto*	43.7(162/371)	+	-	+	+
4. *Cirrhina reba*	18.2(2/11)	-	+	-	-
5. *Heteropneustes fossilus*	14.3(2/14)	+	-	+	-
6. *P. sarana*	13.3(4/30)	+	-	+	-
7. *Labeo calbasu*	8.3(2/24)	+	+	+	-
8. *L. dyochelus*	-	-	-	-	-
9. *Mystus seenghala*	-	-	-	-	-
10. *Notopterus notopterus*	-	-	-	-	-
11. *N. chitala*	-	-	-	-	-
12. *Wallago attu*	-	-	-	-	-
13. *Mystus carasias*	-	-	-	-	-

* UL: Ulcer. EF: Eroded fin. HA: Hemorrhagic area. RS: Raised scale

The various types of pathogens associated with EUS in freshwater fishes in the endemic area of Punjab Pakistan are presented in Table 3. A specific slow growing temperature sensitive fungus, *Saprolegnia* sp. was isolated from all the EUS infected fish species. This fungus was present in form of bunches on the ulcerated skin and with deeply penetrated hyphae (Table-III). Among the parasitic infestation *Lernaea* sp. and *Argulus* sp. were isolated from some of the infected fishes (Table 3). *Trichodina* spp., were also isolated from the gills of infected fish species. Bacterial involvement in this disease was consistently present. *Aeromonas* spp. and *Pseudomonas* spp. were isolated from the diseased fishes (Table 3). Environmental monitoring revealed that the incidence of disease was more severe in water with low alkalinity (70 mg/l of $CaCO_3$) and hardness (75 mg/l of $CaCO_3$). The disease was mostly observed during the winter (November-February), when temperature fall to 10-12 ^{0}C (Table 3).

Table 3. Various type of pathogens associated with EUS in freshwater fishes in the endemic area of Punjab, Pakistan

EUS infected fishes	Pathogens isolated		
	Ecto parasite	Fungal	Bacterial
Culturable fishes			
1. *Labeo rohita*	LS	SS	AH,PS
2. *Cirrhina mrigala*	LS,AS,TS	SS	AH,PS
3. *Catla catla*	LS,AS	SS	AH
Non-culturable fishes			
1. *Labeo calbasu**	-	SS	AH
2. *Cirrhina reba*	LS	SS	AH,PS
3. *Puntius ticto*	LS,TS	SS	PS
4. *Heteropneustes fossilus*	-	SS	PS
5. *Puntius sarana*	LS	SS	AH,PS
6. *Channa punctatus*	TS	SS	AH,PS
7. *Channa straitus*	TS	SS	AH,PS

LS: *Lernaea* sp. AS: *Argulus* sp. TS: *Tricodina* sp. SS: *Saprolagnia* sp. AH: *Aeromonas hydrophila* PS: *Pseudomonas* sp.
*Pathogens were identify in other specimen examiner.

Fish sample analysis (AAHRI, Bangkok)

Case No.1 (*Channa punctatus*): Mycotic granulomae were observed among the myopathy lesion of the affected fish. Some metacercaria found encysted in the hypodermal layer of the skin, muscle and gill filament. *Trichodina spp.* has infected gill of the fish and cause hyperplasia and oedema of epithelial cells.

Case No.2 (*Channa punctatus*): The lesions of this fish sample were in the advance stage as compared to the sample No.I. Many mycotic granulomae were found in the muscle area and also in the mesenteries. *Trichodina spp.* and metacercarial cyst infected

gills of the fish and cause hyperplasia and oedema of epithelial cells. Gill lamellae were joined together.

Discussion

Incidence of EUS in freshwater fishes in the endemic area of Punjab Pakistan is being reported for the first time. This incidence of EUS in three culturable fish species (*C. mrigala, C. catla, L. rohita*) averaged 11.2 % and 7 non-culturable fish species averaged 33.7 %. These results are consistent with the earlier reports of Callinan *et al.* (1997) who reported that out-break of EUS in 18 countries of Asia- Pacific. Over 100 fish species have been recorded as being affected by EUS (Frerichs *et al.* 1988). Highest losses occurred in the snakehead *Channas* sp. (Roberts *et al.* 1989). Other included air breathers that live in swamps, marshes or ditches and many of them experience poor water conditions for most of the year (Supriyadi 1986). Wishwanath *et al.* (1997) reported that in India a total of eight species were consistently affected in freshwater belonging to *Channa* sp. and *Puntius sp.* The Indian major Carps (*C. mrigala, L. rohita* and *C. catla*) also appear susceptible to EUS (Kumer and Day 1992). EUS is believed to have entered India in 1988 and since then has been causing large-scale mortalities in both freshwater and brackish water fish (Das and Das 1993). Since Pakistan share a long eastern border with India, there was a possibility that disease could have been transmitted depending on aquatic environment contamination.

According to Willoughby *et al.* 1995, the essential etiological agent of EUS is an oomyce fungus *Aphanomyces invaders*. The type of ulcers produced in fish from India have been associated with pathogenic fungus of *saprolegnia* sp. (Anoymous 1992). The results of our study indicate that most likely a typical Saprolegnia sp. caused them. In India a broad spectrum of bacterial forms belonging to *Pseudomonas sp., Bacillus sp., Anthrobacter sp., Staphylococcus sp., Micrococcus sp., Actinomycete sp.* and *Aeromonas hydrophila* were isolated from diseased fish sample (Kumar and Day 1992). However, in Pakistan only *Aeromonas sp. and Pseudomonas sp.*, were isolated from the diseased fish in the present study.

Although, Reungprach *et al.* (1983) found no direct relationship of ectoparasite to the occurrences EUS. The present study demonstrated that ectoparasites like *Lernaea sp., Argulus sp. and Tricodina sp.* were also isolated from diseased fish. Perhaps parasite acts either as a pathogen or vector for a pathogen of EUS Roberts *et al.* (1986) . Alternatively parasites may at times induce stress in fish and predispose them to infection.

The incidence of disease appear to be seasonal in nature usually this occurs in real epidemic form after the monsoon season i.e. September onward. This is in agreement with the results of Jhingran (1990), Chinabut *et al.* (1995) and Vish Wanth *et al.* (1997), who has reported that low temperature usually is necessary for EUS out-break. The disease showed a decline from April onwards. This is possibly due to rise of water temperature.

Rodgers and Bruke (1981) opined that rapid seasonal depression of salinity and temperature are important environmental factors that predispose fish to be attacked by

red spot disease. Monitoring at affected sites in Bangladesh, China, India and Lao PDR (Myanmar) during 1988 and 1989, it was found that out-breaks occurred during months with low temperature, (Philipps and Keddie 1990). Low chloride concentrations also make fish less tolerant to environmental toxin (Jhingran 1990). In the present study the alkalinity and hardness were comparatively low in the water samples. It is therefore, logical to believe that these factors might have predisposed the fish with a possible attack by fungus *Saprolegnia* sp.

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