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Pathogenicity of EUS Associated *Aeromonas hydrophila*, Artificially Injected to *Channa striatus* (Bloch)

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Experiments have been conducted with *A. hydrophila,* which was isolated from EUS affected *Mystus cavasius* and injected to *Channa striatus* intraperitoneally and observations were made at regular intervals for 96hrs to study the disease progress. Visible clinical and behavioural symptoms were noticed. Moribund and dead fish were autopsied to observe pathological changes in different organs. The L_D50 value obtained was $2.98x10^3$ cells/ml. The statistical analysis such as regression equation, chi-square, $LogL_D50$ ± standard deviation (SD) and fiducial limits (FL) of the data were recorded as Y= -3.070 + 2.322x, 4.027, 3.4742 ± 0.0651, 2.22×10^3 and 4.00×10^3 cells/ml, respectively. The chi-square value was found to be significant at PÐ 0.05.

Key words : Pathogenicity, EUS *Aeromonas hydrophila*, *Channa striatus.*

Epizootic Ulcerative Syndrome (EUS) is a devasting disease affecting many species of wild and cultured freshwater fishes in asia (Chinabut, 1998). In India also, the EUS has assumed the shape of epizootic proportion and has taken heavy toll of millions of try, fingerling and adult fishes. The first outbreak of EUS was reported from India in the month of May, 1988 from Northeastern region. Thereafter, it spread to Northern and Southern states of the country (Das, 1988; Jhingran and Das, 1990; Pal and Pradhan, 1990; Dastidar and Chakraborty, 1992; Das and Das, 1993; Mohan and Shankar, 1994; Qureshi *et al.,* 1995; Mukherjee, 1996; Lio-po *et al*., 1996; 1998, Mastan, 1998 and Mastan and Qureshi, 1999; 2001; 2001; 2001; 2003; 2004; 2005). EUS is reported to affect over thirty freshwater species of cultured as well as other wild fish population, although it was most notable in Snakehead and Catfishes (Tonguthai, 1985). Wild fish species have been found to be seriously affected by the outbreaks resulting in severe dermal ulceration and large-scale mortality.

Unfortunately, the problem has become complex due to the fact that many vital clues regarding the causative organisms and other factors responsible for EUS outbreaks are yet to be unrevelled and this problem is still evading a plausible solution, despite global efforts (Jhingran and Das, 1990; Das and Das, 1993 and Mastan, 1998). *Aeromonas hydrophila* is consitently associated with EUS affected fish in all the south

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east-Asian countries. Thus, the present study is aimed to test the pathogenicity of *A hydrophila* against *C. striatus* in relation to EUS.

Materials and methods

For the purpose of present study, healthy and disease free fingerlings of *Channa striatus* averaging 12.4 ± 0.28gm weight and 10.40 ± 0.12cm in length were used. They were introduced in aquaria of the size of 90 x 45 x 45cm washed with $KMnO_4$ filled with 100 litres of freshwater each. In each concentration, twenty fishes were used which were acclimatized in laboratory conditions for one week, prior to the experiment. They were fed with pelleted diet once a day approximately 75% of the water was changed everyday Feeding was stopped 24hrs prior to experiment.

Bacterial suspension was prepared by culturing *A. hydrophila* on TSA plates at 30^0C temperature for 24hrs and harvesting them with 500ml of 0.85% physiological saline. Colony forming unit was then determined by plating 10 fold dilution series. Later, it was diluted with distilled water to make a cell number in the powers of 10^3/ml and used for exposure to fish. The counting of cells were done with the help of haemocytometer. Bacterial strain used in this study was originally isolated from naturally EUS affected fishes. Symptoms and mortality was recorded upto 96hrs after injection. Each experiment was carried in triplicate sets.

RESULTS AND DISCUSSION

Fingerlings of *Channa striatus,* experimentally injected with the challenge organism, *A hydrophila*, revealed the following behavioural and clinical symptoms. The fishes were inactive and were lying at the bottom showing loss of movement. Sometimes, they swam in circling manner, sometimes upside-down and sometimes in flating position. Some fishes were seen lying down on the bottom of aquaria before death.

Other symptoms shown by them were relative more mucus secretion and the appearance of haemorrhages on the general surface of body. Haemorrhages were seen in all the fishes within 18hrs after injection. Superficial ulcers developed at the point of injection. In some fishes scales got

Table 1. Showing the results of experimental infection produced in *Channa straitus* with *Aeromonas hydrophila,* isolated from EUS affected fishes

S. No.	Conc. in (cells/ml)	Symptoms observed	Mortality	Regression equation (Y=a+bx)	Chi-square (X^2(n-2)	L_D50	$LogL_D50$±SD	95% FL (cells/ml)
1.	1.40×10^3	-	30	Y=-3.070+2.322x	4.027*	2.98×10^3	3.4742± 0.0651	LL : 2.22×10^3 UL¨4.0×10^3
2	2.52×10^3	Haemorrhages	45					
3	3.50×10^3	Haemorrhages	65					
4	7.60×10^3	Haemorrhages	80					
5	Control	-	0					

FL = Fiducial limit; LL : Lower limit; UL : Upper limit
*Value is significant P Ð 0.05

raised and became loose while in others they fell down. Severity of symptoms increased with the increase in bacterial concentration.

Table 1 exhibits results of statistical analysis of data for pathogenicity of *A. hydrophila* against fingerlings of *Channa* striatus. When four different concentrations of *a. hydrophila* suspension, ranging from 1.4×10^3 to 7.6×10^3 cells/ml were inoculated to *Channa striatus,* they caused 30 to 80% mortality within 96hrs after injection. The L_D50 value obtained was $2.98x10^3$ cells/ml. the statistical analysis such as regression equation, chi-square, Log L_D50 Standard deviation (SD) and fiducial limits (FL) of the data were recorded as Y = -3.070 + 2.322 x, 4.027, 3.4742 ± 0.0651, 2.22×10^3 and 4.00×10^3 respectively. The chi-square value was found to be significant at P Ð 0.05 .

Though, the fish and the pathogens live together in an aquatic environment, the disease manifestation occurs only when the susceptible fish and the virulent pathogen happen to meet under certain favourable environment conditions. In earlier reports of bacteria associated with fish diseases, the workers have made little efforts to differentiate between primary and secondary role of bacteria. In the present investigation, experimental infection trial were conducted with *A. hydrophila,* which was isolated from naturally EUS affected fishes. It was inoculated intraperitoneally in the fingerlings of *C. striatus*. The pathogenicity tests showed that *A. hydrophila* was pathogenic to fish. This observation is in agreement with findings of Pradhan and Pal (1990), Ali and Tamuli (1990), Singh *et al.,* (1991), Lio-Po et al., (1992) and Karunasagar (1995). Pal and Pradhan (1990) have reported that *A. hydrophila* and *P. fluorescens* could kill the host fish more rapidly than other species of bacteria *A. hydrophila* is found to be the most destructive pathogen causing infection even within eighteen hours after injection. This finding conforms wity the work of Shankar (1995) who reported that the infection caused by *A. hydrophila* in *Cyprinus carpio* resulted in mass mortality. It has been observed that inoculation of *A. hydrophila* produced symptoms of EUS such as the appearance of small raised haemorrhagic areas on the body surface, erratic swimming movements, staying of fish near the surface layer of water with head projecting out of it, lethargic condition and non-feeding tendency. In the present investigation, L_D50 value was recded at $2.98x10^3$ cell/ml. the recorded fiducial lower and upper limits are $2.22x10^3$ and $4.0x10^3$cells/ml, respectively. Chi-square value is found significant in experimental fishes as compared to control ones. The results of the present investigation reveals that *A. hydrophila* may be considered as potential pathogen for producing EUS in fishes.

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