

Summary

The present study dealt with the "Evaluation of the role of physico-chemical and biological factors on the outbreak of epizootic ulcerative syndrome of fish" consisting of (1) Studies on some physico-chemical parameters of pond water in epizootic ulcerative syndrome affected areas of North Bengal, (2) Histopathological studies of naturally infected fish, (3) Studies on RBC count and haemoglobin content of naturally infected fish, (4) Isolation, characterization and identification of bacteria from infected fish, (5) Isolation of fungus from infected fish, (6) Pathogenicity of bacteria isolated from EUS affected fish, (7) Histopathology of fish experimentally infected by the isolate, *A. hydrophila*, (8) Histopathology of fish experimentally infected with the fungus isolate, *Aphanomyces* sp. (9) Studies on RBC count and haemoglobin content of fish experimentally infected with bacteria isolated from EUS affected fish.

In India, EUS first occurred in May 1988. From the beginning, it affected a wide variety of fish species in both wild and cultured waters. By the year 1993, the disease spread all over India barring a few states. In some areas of North Bengal, the disease recurred every year.

The experiments conducted during the investigation has been presented in three broad sections. The first section deals with studies on the physico-chemical parameters of pond water (section 4.1). The second section (4.2-4.5) deals with the signs, histopathological and haematological observations of naturally infected fish. The third section (4.6-4.12) deals with the isolation of bacteria and fungus and their characterization and pathogenicity. This section also includes the histopathological and haematological observation of fish experimentally infected by the bacterial and fungal isolates.

Three ponds, located at Malbazar, Singhimari and Salbari where EUS occurred every year, were selected for the study on the physico-chemical parameters of the pond water. The results showed that in all the three ponds significant declining trend of the dissolved oxygen content, hardness and total alkalinity, were observed during the month of September-October. The water temperature remained low during October - March and the water also remained very slightly acidic.

Altogether, 234 infected fish namely: *C. punctatus*, *C. gachua*, *C. striata*, *Mystus* sp., *C. batrachus*, *L. rohita*, *L. bata*, *C. catla*, *C. mrigala*, *Puntius* sp. were collected. Out of 234 infected fish collected 40 were with initial stage of ulcer, 44 with moderate ulcer and 150 with advanced stage of ulcer.

Histopathological studies were conducted on EUS affected fish, *C. mrigala*, *C. catla*, *Puntius* sp., *C. gachua* and *C. batrachus*. The Grocott methenamine silver stain and Periodic acid-schiff stain stained section of ulcer showed the presence of fungal hyphae while the H&E stained sections showed loss of epidermis. Necrosis of muscle, granulomatous changes and blood capillary infiltration in the dermal and subdermal layers at the site of ulcers were prominent changes. Liver showed vacuolation of hepatocytes, necrosis and infiltration of blood capillaries. In the kidney, vacuolation, tubular degeneration and haemorrhage were the major changes. Spleen also showed degenerative changes.

Studies were conducted on the changes in erythrocyte morphology, total erythrocyte count (TEC) and haemoglobin (Hb) content of naturally infected *C. mrigala* and *C. gachua*. Significant reduction of TEC and Hb content were observed in infected fish compared to healthy fish.

Bacteria were isolated from ulcers of naturally infected *Puntius* sp. *C. gachua* and *C. mrigala*. Among the isolates of *Puntius* sp. one belonged to the genus *Pseudomonas*, one belonged to the genus *Aeromonas*, one belonged to the genus *Micrococcus* and one belonged to the genus *Moraxella*. Among the isolates of *C. gachua* one belonged to the genus *Aeromonas* and one belonged to the genus *Micrococcus*. Among the isolates of *C. mrigala* one belonged to the genus *Aeromonas* and one belonged to the genus *Micrococcus*.

Fungus was isolated from the ulcers of naturally infected *C. mrigala* and *C. gachua*. The isolated aseptate fungus was identified as *Aphanomyces* sp. by its characteristic zoosporangia which was not wider than the hyphae and the ball of spores at the tip of the sporangium. A single row of primary zoospores

was found within the zoosporangia. In culture media, the fungal hyphae became slender. The isolated fungus did not grow at 37°C.

Pathogenicity studies with the bacterial isolates showed that only some strains of bacteria belonging to the genus *Aeromonas* and *Pseudomonas* were virulent. Isolates P₃, C₂ and M₂ (*Aeromonas* sp.) and P₁ (*Pseudomonas* sp.) were found to be pathogenic. Isolates belonging to other genera were non pathogenic.

Histopathological studies were also conducted on *H. fossilis* and *C. punctatus* after intramuscular injection with P₃ bacterial isolate. Results showed the complete loss of epidermis and development of haemorrhagic spot at the centre of the lesion. The dermal layer had slight necrotic changes. In the liver, vacuolation, necrosis and infiltration of blood capillaries were observed. Tubular degeneration and vacuolation of tubular cells were observed in the kidney.

Pathogenicity studies with the zoospores of isolated fungus *Aphanomyces* sp. showed that the isolated fungus was pathogenic.

Histopathological studies were conducted on *C. punctatus* after intramuscular injection with the zoospores of isolated *Aphanomyces* sp. The loss of epidermis and dermis of the skin of ulcerated area were noted but the dermis showed severe changes wherever the fungus was present. Severe myonecrosis and granuloma formation were found. Aseptate fungal hyphae were found in the dermis and underlying musculature. In the liver, vacuolation and haemorrhages were observed. Necrotic changes were observed in the kidney. No evidence of fungal hyphae were observed in the liver and kidney.

Studies were conducted on the morphological changes of erythrocyte, total erythrocyte count (TEC) and haemoglobin (Hb) content of *H. fossilis* after intraperitoneal injection with pathogenic bacterial isolates. Vacuolation in the cytoplasm of the erythrocyte, disintegration of erythrocyte and significant lowering of TEC and Hb content were observed in inoculated fish. In the control fish no remarkable changes were observed.

Declining temperature and pH of the water of the ponds might cause severe stress and immunosuppression which ultimately make fish susceptible to pathogens. Bacteria, *Aeromonas* spp. and *Pseudomonas* sp., and fungi, *Aphanomyces* sp. have important roles in the manifestation of the disease in experimental fish. *Aphanomyces* sp. induced typical granulomatous changes in experimental fish and caused 30% mortality which is lesser than that of during natural outbreak of EUS. Studies on immune response of fish with respect to changes in water quality and temperature are necessary. It is also necessary to evaluate the role of virus on the outbreak of EUS in this area of North Bengal.