

Sporadic occurrence of Epizootic ulcerative syndrome outbreaks under post flood conditions in Kerala

K. J. Reshma*, T. G. Sumithra, Anusree V. Nair, P. Sayooj, P. V. Amala and Sanil N. K.
ICAR-Central Marine Fisheries Research Institute, Kochi-682 018, Kerala

*Email: reshjanardhan@gmail.com

Epizootic ulcerative syndrome (EUS) is considered to be an infection with an oomycete known as *Aphanomyces invadans*, commonly known as water molds causing

heavy mortalities in wild and in farm fish. Large-scale fish mortality waters especially in the flood-affected fish farms in Kerala, following heavy rainfall and flood in

August 2018 were reported. Subsequently, several fish disease cases were reported to ICAR-CMFRI by farmers, with characteristics of the invasive, tissue-destructive stages of EUS (Fig.1). In such cases reported, several species tested positive for EUS including *Channa bleheri*, and *C. diplogramma*. The affected fishes showed clinical symptoms like lethargy and lateral recumbency in rearing facilities. In some fish, the caudal peduncle, caudal fin or dorsal fins were severely eroded, and deep ulcers exposing the underlying musculature were also observed.

The infected fishes were transported to ICAR-CMFRI and routine disease investigation procedures were carried out in microbiology laboratory. The submitted fish were observed for gross pathological changes. The systematic evaluation of the infected fishes showed different pathological features like haemorrhages on the operculum, head, ventral abdomen, vent and posterior to pelvic fin and base of fins. Further, they showed loss

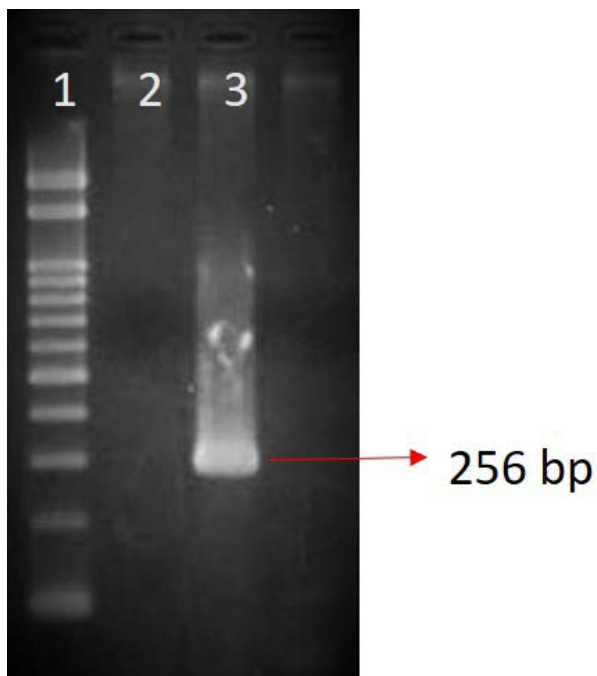


Fig. 1. Agarose gel electrophoresis profile of PCR-amplified for EUS: Lane 1: 1 kb DNA ladder; Lane 2: Negative control Lane 3: Infected fish sample. A special band about of 256 bp showed the positive results

of body scales, blackish discolouration at the tip of gills, empty gut, white cotton like fungal growth on ulcers and the left posterior side of the head etc. In severely affected fish, large, deep red-colored ulcers were observed. Blood, liver, kidney, brain and spleen samples were collected aseptically and screened for fish pathogens. Further, the

fungal growth seen on the ulcers of affected fish was directly taken for molecular identification. The direct microscopical examination of the squash preparations from affected tissues shown the presence of clusters of encysted primary zoospores on non-septate hyphae of *Aphanomyces invadans*. Confirmatory diagnosis was done through PCR using extracts from affected tissues. Molecular confirmation indicated amplicons of the desired size (ITS region, amplicon size 256 bp) (Fig. 2), which were consistent in all suspected samples from affected hosts. Although viral screening were negative, bacteriological investigation of blood and other internal organs discovered the co- infection with bacteria like *Sphingomonas paucimobilis*.

A number of predisposing factors and postulates were attributed to such expansion of hosts susceptible to EUS. However, previous reports worldwide manifests that temperatures drop are suspected to have played a critical role in increasing susceptibility of fish to infection by *A. invadans*. Lower water temperatures on post flood conditions may resulted in a retarded immune response in fish. Heavy rainfall and soils which are either naturally acidic or disturbed by agriculture or residential development may lead to a lowering of water pH, which might have caused skin trauma, providing a portal of entry for infective zoospores. Further, sudden changes in hydrographic parameters of estuarine water next to heavy freshwater influx after flood might have created stressful conditions for brackish-water fishes, paving the way for the spread of EUS, and concomitant bacterial infections.

The sporadic outbreaks of EUS post flood season and report of this destructive pathogen associated mortalities in closed and isolated facilities like ornamental facilities and negatively certified imported fishes suggests enzootic occurrence of this pathogen post flood seasons. Further, the expansion of hosts and co-infection with new bacterial pathogens should also viewed seriously, considering the 100% mortality in case of EUS outbreaks. In this context, the invasive nature of EUS and its apparent pathogenicity to many endemic host fish species endorse that EUS poses a substantial threat to other fishes, especially at the climate driven risks of floods in Indian continent. Surveillance, pathology testing, more vigilant reporting of suspected outbreaks and further investigation upon its spread and impact on wild and cultured fish has to be given priority, and this will assist in the development of comprehensive biosecurity practices against EUS spread in present scenario.