



## Note

# Epizootic ulcerative syndrome (EUS) in bata, *Labeo bata* (Hamilton 1822) from Tripura, India

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## ABSTRACT

The occurrence of epizootic ulcerative syndrome (EUS) in bata (*Labeo bata*) and the infectivity and role of *Aphanomyces invadans* in the aetiology of ulcerative condition were investigated in the present study. Live fish (*L. bata*) samples with ulcerations were collected from a local fish farm in Tripura and were acclimatised in laboratory tanks with proper management. Case histories, clinical and gross signs of the sampled fishes were recorded. Histopathological study of muscle and liver tissue from the ulcerated fish showed the presence of oomycete hyphae and granulomas. Oomycete isolation was done from the affected muscles and was identified as *Aphanomyces invadans* based on colony morphology, growth and microscopic morphology. The results suggest that the ulcerative condition in bata was due to EUS associated with *A. invadans*.

Keywords: *Aphanomyces invadans*, Epizootic ulcerative syndrome (EUS), *Labeo bata*, Histopathology

Epizootic ulcerative syndrome (EUS) is one of the most destructive diseases affecting a large number of wild and cultured freshwater and estuarine finfish species. The aetiological agent of EUS is *Aphanomyces invadans*, an oomycete that has spread across the Asia-Pacific region (Lilley *et al.*, 1997). Outbreaks of EUS have been reported from several southern, eastern and north-eastern states of India (Das, 1997; Vishwanath *et al.*, 1997). Available literature indicates that EUS outbreaks generally occur during winter months, coinciding with a fall in water temperature (Lilley *et al.*, 1998). The present study was conducted in Tripura, one of the north-eastern states of India, where the temperature is around 20-25°C during winter months, which is ideal for EUS outbreaks. Information available in India is largely confined to reports of prevalence of EUS and associated mortality along with histopathological demonstrations (Vishwanath *et al.*, 1997, 1998; Kar *et al.*, 2000; Pradhan *et al.*, 2007). Little attention has been given to the isolation and identification of *A. invadans* from the EUS-affected fish samples. The present study was designed to isolate and identify *A. invadans* from bata (*Labeo bata*) affected with EUS like ulceration.

Ulcerated bata (mean weight 80 g) were obtained from a local fish farm in Tripura, India. The fishes

were immediately brought to the laboratory and were acclimatised in 1000 l indoor circular tanks holding dechlorinated tap water. The fish were acclimatised at ambient temperature (23-26°C) with necessary aeration and were fed twice daily with rice bran and mustard oil cake in the ratio of 1:1. The basic physico-chemical parameters of water were measured systematically throughout the period of acclimatisation and were maintained at optimum levels (dissolved oxygen: 6.88±0.56 mg l<sup>-1</sup>; pH: 7.14±0.77; ammonia: 0.029±0.011 mg l<sup>-1</sup>; nitrite: 0.016±0.01 mg l<sup>-1</sup>). Periodical exchange of water was done to remove the waste feed and faecal material.

Isolation of *A. invadans* from ulcerated bata was done following the method of Lilley *et al.* (1998). Fish were killed by decapitation and pinned to a dissecting board, with the lesions uppermost. The scales around the lesions were removed and the underlying skin was seared with a red hot spatula so as to sterilise the surface. Using a sterile scalpel blade and forceps, the underlying muscles were exposed, inside a laminar flow cabinet. Pieces of muscles (2-4 mm<sup>3</sup> size) were excised aseptically and placed in petridish containing glucose-peptone-penstrep broth (GP-PenStrep broth; Willoughby and Roberts, 1994; Lilley *et al.*, 1998). Inoculated media were incubated at 25°C and were examined under an inverted microscope

within 12 h. Emerging hyphal tips were repeatedly transferred to fresh plates of GP-PenStrep agar until the cultures were free from bacterial contaminations. The fungus was subcultured, by aseptically cutting a block of agar (approximately 3-4 mm in dia) from the periphery of a colony and placing this upside down in a petridish containing fresh agar.

For inducing sporulation, an agar plug (3-4 mm dia) of actively growing mycelium was placed on a petridish containing glucose-peptone-yeast broth (GPY-broth; Lilley *et al.*, 1998) and it was incubated for 4 days at approximately 20°C. Autoclaved pond water (APW) was prepared after mixing and autoclaving one part pond water (from the infected pond) with two parts of distilled water. The agar plug was washed out of the resulting mat by sequential transfer through 5 petridishes containing APW, and leaving overnight in APW at 20°C. After 12 h, achlyoid clusters of primary cysts formed and the release of motile secondary zoospores was apparent under the microscope. Tissues, such as muscle and liver from the ulcerated bata, were used for histopathological examination. Standard procedures for histopathology was followed for tissue fixation, processing, sectioning and staining.

The present study provides the first direct evidence of *A. invadans* as the primary aetiological agent of ulcerative condition in *L. bata*. Proper diagnosis of EUS is important in order to avoid any confusion with other ulcerative conditions. The clinical signs and gross pathology of any disease form the basic step in disease diagnosis. According to the information provided by the farmer, the early lesions of the affected fish were pin - head sized red coloured spots without any ulceration

and the affected fish showed abnormal swimming behaviour. During the sample collection for the present study, the lesions were at moderately advanced stage with ulceration covering a larger area with or without sloughing of scales (Fig. 1). Some affected fish had more advanced lesions in the form of circular or open ulcers with loss of dermis and scales. Similar type of external pathological changes have been reported from EUS affected fish in India (Kumar *et al.*, 1991; Karunasagar and Karunasagar, 1994; Das, 1997; Viswanath *et al.*, 1997) and other countries (Lilley *et al.*, 1998). Sampling of ulcerated bata for the present study was done during the winter month, which is considered as ideal environmental condition for occurrence of EUS (Lilley *et al.*, 1998).

However, clinical sign and gross pathology alone cannot confirm the positive diagnosis for a disease condition. In case of EUS, the typical diagnosis can be achieved by demonstrating the presence of fungal hyphae and mycotic granuloma in histological sections and isolating *A. invadans* from affected tissues (Lilley *et al.*, 1998). Histopathological studies conducted on ulcerated bata showed identical histopathological manifestations (Fig. 2) as reported in other fish species (Roberts *et al.*, 1994; Vishwanath *et al.*, 1997; Pradhan *et al.*, 2007). Histopathological results of muscle and liver tissue showed that a specific type of oomycete was involved in naturally infected bata which was highly invasive in nature. The presence of oomycete hyphae in liver indicates the ability of the hyphal tips to proliferate rapidly. There is evidence for the invasive ability and presence of hyphae in the internal organs of some freshwater and brackishwater fish (Roberts *et al.*, 1992; Vishwanath *et al.*, 1998) during natural outbreaks. In the present study, mycotic granulomas and other pathological manifestation like



Fig. 1. (a) EUS-affected *Labeo bata* with typical dermal ulceration on different parts of the body surface, (b) Lesions causing destruction of muscle tissues near caudal peduncle with brown coloured fungus like growth.

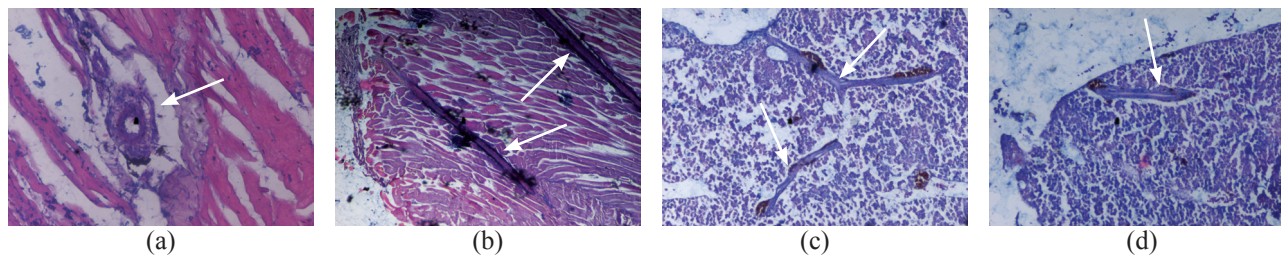


Fig. 2. (a) Muscle section of *Labeo bata* showing granuloma formation (arrow) with myonecrosis around the granuloma (H&E, ×200), (b) Fungal hyphae (arrow) in muscle section of bata (H&E, ×200), (c) Fungal hyphae (arrow) penetrating across the liver of naturally infected bata (H&E, ×200), (d). Presence of fungal hyphae encapsulated by granuloma (arrow) in the liver of bata (H&E, ×200).

myonecrosis, was noted to occur within the tissue section of muscle and liver. A large number of granulomas have been demonstrated in different organs including muscle and liver of several fish (Chinabut, 1990; Wada *et al.*, 1994; Ahmed and Hoque, 1999; Sosa *et al.*, 2007; Dahail *et al.*, 2008).

Isolation of *A. invadans* from the affected fish tissues was successfully done during the present study. A specific type of non-septate, broad, sparsely branching hyphae grew from the muscle sections in petridish containing the isolation medium. Following sporulation, there was formation of filamentous zoosporangia, indistinguishable from hyphae and achlyoid cluster of primary zoospores (Fig. 3). The formation of achlyoid cluster of primary zoospores at the mouth of the zoosporangium and the hyphal structure were morphologically similar to *A. invadans* as described by other workers (Willoughby, 1994; Lilley *et al.*, 1998).

Furthermore, colonial morphology, growth and microscopic morphology of the present isolates were consistent with those of *A. invadans* isolates from Philippine fish (Callinan *et al.*, 1995) and Thailand fish (Roberts *et al.*, 1993). Like the Philippine isolates, the present isolates typically had 1 or 2 lateral evacuation tubes per sporangium and not 4 described as typical for the Thai isolates. No other fungi, morphologically consistent with those present in ulcers of bata, were recovered in cultures.

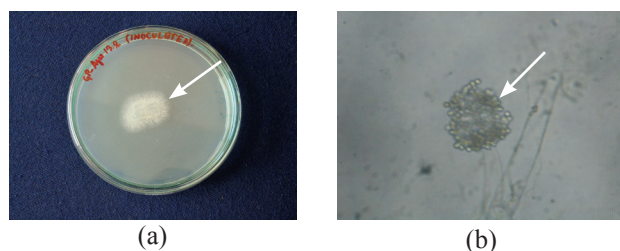


Fig. 3. (a) Glucose-peptone agar plate (GP-agar) with the growth of broad, non-septate, and sparsely branching oomycete hyphae (arrow), (b) Zoosporangia formation on the tip of the oomycete hyphae (arrow).

The results of the present study clearly indicated occurrence of EUS in *L. bata* with fungal aetiology. Mycotic granulomatosis response against the invading fungal pathogen and presence of fungal hyphae in the liver sections indicated highly invasive ability of the pathogen.

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