

This issue contains papers dealing with causative agents for epizootic ulcerative syndrome in finfish, ecology and feeding habits of some aquaculture species and hybridization as a method for increasing growth in groupers, all elements with a potential for increasing aquaculture production. The emphasis today is on research aimed not only at increasing production but also sustaining the ecological integrity of aquatic resources for sustained production. Discussions in symposiums held recently and planned for the near future highlight this emphasis. I am sure that network members are giving due importance to this aspect in their research.

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Is Epizootic Ulcerative Syndrome (EUS) Specific Fungus of Fishes a Primary Pathogen? - An Opinion

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Abstract

Earlier findings on epizootic ulcerative syndrome (EUS) and the present observation of the authors on transmission of EUS to snakehead (*Channa sp.*) without skin damage provide evidence to suggest that the invasive fungus associated with EUS is a primary pathogen.

Introduction

A highly invasive, aseptate and specific slow growing fungus originally described as *Aphanomyces invaderis* (Willoughby et al. 1995), now listed as *Aphanomyces invadans* in the Index of Fungi (Lilley and Roberts 1997) is the main aetiological component of epizootic ulcerative syndrome (EUS) of fresh and brackishwater fish in Asia.

This fungus is pathogenically and culturally identical and similar to *Aphanomyces* isolates from fish suffering from red spot disease (RSD) in Australia and mycotic granulomatosis (MG) in Japan (Callinan et al. 1995; Lilley and Roberts 1997; Lilley et al. 1997).

Research has gone into the reproduction of the disease using different transmission routes. Hatai et al. (1977) reproduced typical MG

lesions in ayu and goldfish by injecting fungal hyphae intramuscularly. Introduction of spores and mycelium of *Aphanomyces* below the dermis of snakehead fish have been shown to produce the advanced hemorrhagic lesions and high mortalities similar to that experienced during natural EUS outbreaks (Roberts et al. 1993; Chinabut et al. 1995). Typical RSD lesions in mullets have been reproduced by

exposing fish with experimentally abraded areas of skin to *Aphanomyces zoospores* (Callinan 1994). Using similar techniques, typical EUS lesions in African catfish (*Clarias gariepinus*) in Indonesia and striped snakehead (*Channa striatus*) in the Philippines have been produced (Callinan 1994).

Aphanomyces species recovered from affected fish have been proposed as primary causative agents for MG (Hatai et al. 1977) and as the necessary cause for RSD (Fraser et al. 1992). Considerable knowledge has been generated on the fungal component of EUS, but it is believed that the fungal invasion is secondary to primary skin damage and or immunosuppression. Lilley and Roberts (1997) consider that the fungus cannot be regarded to be the primary cause of EUS unless the infective zoospore stage can be shown to breach the fish epithelium. A major hurdle in proposing a primary role for fungus in EUS has been the inability to transmit the disease to healthy fish using a natural transmission route.

Infection by a route other than subcutaneous inoculation has been unsuccessful (Roberts 1994). So far, bath challenges have only succeeded when scraped or artificially injured fish were used (Callinan 1994) and healthy fish have not been shown to be invaded by fungal hyphae or spores in suspension (Chinabut et al. 1995). Balasuriya et al. (1990) have demonstrated transmission of EUS to healthy native fish (*Channa striatus* and *C. punctatus*) through direct contact with water from an EUS-affected environment or feeding with EUS-affected fish. Subasinghe (1993) has also shown transmission of EUS to naive snakeheads while studying the effects of controlled infections of *Trichodina* sp. on transmission of EUS. Cruz-Lacierda and Shariff (1995) achieved 100% transmission of EUS to naive snakeheads by cohabitation with EU positive snakeheads in an EUS-enzootic environment and exposure to water

from an EUS-enzootic environment alone. They observed initial signs of EUS after 9 days, progressing to advanced stages in 10-16 days. However, in all these studies, only the gross clinical pathology was used to assess successful transmission and the histopathological diagnostic features of EUS lesions were not described.

The findings on epidemiological aspects of EUS (Mohan and Shankar 1994), role of fungal induced pathology in EUS (Mohan and Shankar 1995), clinical and histopathological characterization of different types of EUS lesions and evidence for involvement of fungus from a very early stage of the disease (Vishwanath et al. 1997a), identification of mycotic granulomatosis and seasonality as the key features of EUS (Vishwanath et al. 1997b) and demonstration of the highly invasive abilities of EUS fungus in tissues like bone, gizzard, spinal cord (Vishwanath et al. 1998) provide indications that the fungus can invade the healthy skin of fishes like snakeheads, silver barb (*Barbodes* sp.) and mullets and cause extensive tissue damage resulting in the formation of the characteristic hemorrhagic ulcers.

This paper presents the observations of the authors (not an experimental study) on transmission of EUS to snakehead (*Channa* sp.) without skin damage through cohabitation. An attempt has been made to analyze the different transmission experiments conducted thus far and to provide arguments to suggest that the invasive fungus recorded in the tissue sections is the likely primary pathogen of EUS.

Observations

Between December 1997 and January 1998, there was an outbreak of EUS in a snakehead fish farm in Bethamangala, Kolar District of Karnataka, India. About 1 500 fry of *Channa* sp. were stocked in a pond with an area of 1 200 m² dur-

ing October 1997. The pond also had a large number of fry of *Mystus* sp. and other weed fishes. Gross EUS lesions started appearing on snakeheads from the first week of December 1997, coinciding with decreasing water temperature (16-22°C). By the first week of January 1998, the majority of the snakeheads with advanced open dermal ulcers had died. At this time, there was also low level mortality of *Mystus* fry, but the surviving *Mystus* sp. had hemorrhagic lesions with superficial mycelium extending into the water. Morphologically these mycelium appeared very similar to pathogenic *Aphanomyces* mycelium described by Roberts et al. (1993). Samples of snakeheads and *Mystus* sp. collected from the farm at this time had the characteristic gross and histopathological diagnostic features of EUS.

One snakehead with open dermal ulcers and about 30 fry of *Mystus* sp., with and without hemorrhagic lesions were collected live on 15 January 1998 and transported live to Mangalore in oxygen packing. The fish were held in the pond water in a bucket for nearly two hours before oxygen packing, during which time most of the pond water was replaced with clean tap water. The fish were in the transportation medium for nearly 18 hrs. They were transferred to a cement tub (0.8 m³) filled with clean well water on the morning of 16 January 1998. By the afternoon, the snakehead and around 10 *Mystus* were moribund and were sampled for histopathological examination. All the fishes were found to be histopathologically positive for EUS.

On the next day, three advanced fingerlings (9 cm in length) of *Channa* sp. and two each of rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) fingerlings (10 cm) were released in the cement tub which had the remaining fry of *Mystus* sp. The fingerlings of *Channa* sp. had been collected as

small fry from the paddy fields of Mangalore during the previous post-monsoon season (October-November 1997) and were being maintained in cement cisterns for a different experimental purpose. There has been no incidence of EUS in the freshwater systems of this region for the preceding three years. The introduced fishes and the remaining *Mystus* sp. were observed daily until the middle of February 1998. Water in the cement tub was not changed and the fishes were not fed. The average daily water temperature during this period ranged from 22 to 28°C.

Between day 9 and 10, gross EUS-like lesions appeared on all the three snakehead fingerlings and by day 15 one snakehead with a well developed dermal ulcer was moribund, and was sampled. The other two snakeheads with open dermal ulcers became moribund by day 18 and were sampled for histopathology. Interestingly, all the three snakeheads had the characteristic histopathological diagnostic features of EUS.

During the 18 days of observation, about 60% of *Mystus* sp. (12) died with hemorrhagic lesions while the rest (8) died gradually over the next 10 days. Samples of *Mystus* sp. consistently showed diagnostic features of EUS. At the end of the 28-day observation period, both *L. rohita* and *C. mrigala* fingerlings were found to be healthy with no signs of gross ulcerative lesions. It is difficult to explain why *L. rohita* and *C. mrigala* were not invaded by the pathogen. In the last 6 years, the authors have not found any histopathological evidence for EUS in major Indian carps in natural outbreaks in Karnataka (Vishwanath et al. 1997b).

Discussion

Irrespective of how and from where the infective stages (zoospore, mycelium) come, it is clear that they are able to invade

the skin of *Channa* sp. unaided and produce the clinical characteristics and histopathological features of EUS. Our present observation provides clinical and histopathological evidence for 100% transmission of EUS to *Channa* sp. by cohabitation, without any skin damage. The time taken for ulcer development and subsequent mortality compares well with earlier transmission studies using the inoculation route (Chinabut et al. 1995) and the cohabitation route (Cruz-Lacerida and Shariff 1995) under somewhat similar temperature regimes.

The present observation highlights the need to examine the underlying reasons as to why earlier bath challenge studies with spores or mycelium have failed to induce the disease in healthy fish with undamaged skin. Several points come to light. Lilley and Roberts (1997) suggested that many trials would have used fish from areas that have been exposed to EUS and may have some innate resistance to fungal invasion. The experience with *Channa* sp. fingerlings which were not exposed to EUS pathogen or EUS-affected water supports the argument of Lilley and Roberts (1997). Secondly, the infectivity aspect of mycelium and/or spores obtained under laboratory conditions needs to be carefully assessed. It may be that the spores and mycelium produced under culture conditions are less infective compared to the stages present in the environment (water, soil, fish) during an outbreak. Variations have been observed with regard to the morphology of the hyphae between fungus in lesions and culture specimens. In addition, reproductive structures have never been observed within the tissues or on the surface of ulcers, although it is a feature of the fungus when grown in fluid culture (Callinan 1994; Roberts 1994). The third point that should be looked into is the optimum density of infective spores or mycelium required to induce an infection in a healthy fish.

Conclusion

The present observation of 100% transmission of EUS to *Channa* sp. clearly indicates that the infective stage of the fungus is able to invade the fish skin unaided. Based on knowledge on the involvement of fungus from a very early stage of the disease (Vishwanath et al. 1997a), the highly invasive ability of the fungal pathogen to pass through tissues like bone, gizzard and spinal cord (Vishwanath et al. 1998) and the present observation on 100% transmission to healthy fish through a more natural route, it seems logical to think that the fungal pathogen responsible for the extensive tissue damage and the resulting clinical dermal ulcers in EUS is a true preliminary pathogen.

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