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# Susceptibility of Fingerlings of Indian Major Carps to *Aphanomyces invadans*

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## Abstract

Epizootic ulcerative syndrome (EUS) is a serious disease of tropical fish. Although reported in over 100 species of fish, its impact on the widely cultured Indian major carps has not been properly assessed. Using artificial infection tests with *Aphanomyces invadans*, the etiological agent of EUS, the present investigation examined the disease susceptibility of fingerlings of Indian major carps. The disease susceptibility experiments indicated that there was 100% mortality in the case of all the three species of Indian major carps within an experimental period of 12 days. Histopathological examination of all the moribund fish indicated extensive myonecrosis in large areas of myotome and severe necrotic lesions in almost all internal organs due to the invasive spread of the hyphae. The massive internal necrotic pathology was reflected in the form of severe gross lesions and was always associated with mortalities. From the present study, it is confirmed that fingerlings of Indian major carps are highly susceptible to *A. invadans* infection. This is the first ever experimental infection studies with *A. invadans* in Indian major carps.

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Epizootic ulcerative syndrome (EUS) is one of the most destructive diseases of fresh and brackish water farmed and wild fish in the Asia-Pacific region. The necessary causative agent of EUS is an oomycete fungus, *Aphanomyces invadans* (Baldock et al. 2005). More than 100 fish species are reported to be affected by the disease (Lilley et al. 1998). However, its impact on the widely cultured Indian major carps has not been properly assessed. Despite several reports from India of Indian major carps being affected by EUS (Das 1997; Das and Mukherjee 1998), the diagnostic features confirming the OIE recommended diagnostic features of EUS (OIE 2003) have not been demonstrated in their reports. Interestingly, Indian major carps present in many water bodies during natural EUS outbreaks in several Southern Indian states such as Karnataka (Vishwanath et al. 1997a; 1997b; 1998) and Tamilnadu (Jayaraman 1991) were found to be unaffected. It has been reported by Chinabut and Roberts (1999) that there is an anomaly with respect to the susceptibility of Indian major carps in India i.e. the Indian major carps appear resistant in the south but not in the north. However, there are no controlled infection studies to confirm these observations. The present investigation using artificial infection tests with *A. invadans*, examined the disease susceptibility of fingerlings of all the three species of Indian major carps, catla, *Catla catla*, rohu, *Labeo rohita* and mrigal, *Cirrhinus mrigala* (Cyprinidae) in one of the southern states (Karnataka) in India.

Indian major carps used in the experiment were procured from the Karnataka State Government Bhadra reservoir project fish hatchery at fry stage (15 days old) and were reared in the fish farm of the College of Fisheries, Mangalore, India. After one month (fingerling stage) of rearing 20 fingerlings of catla, rohu and mrigal each (averaging 7.1±0.22, 7.0±0.24 and 7.2±0.15 cm, respectively) were used for the artificial infection test. All the fish species were divided into experimental and control groups having equal numbers of fish. Each experimental fish was injected intramuscularly with 0.1 ml of spore suspension as described by Chinabut et al. (1995) while the control fish group received 0.1 ml of autoclaved pond water (APW). Suspension of motile secondary zoospores of *A. invadans* was prepared as described by Lilley et al. (1998). After injection, each fish species of experimental and control groups were kept separately, in 500 l capacity fiberglass tubs containing 400 l of water. Aeration was maintained with replenishment of 50% of water daily. Water temperature of the experimental tanks ranged from 26 to 29°C as measured twice daily in the morning and in the evening. The mortality pattern was recorded daily up to 12 days post challenge. Specific mortalities were confirmed through his-

tology and re-isolation of *A. invadans* from the muscle tissue as described by Lilley et al. (1998).

The mortality pattern of fingerlings of catla, rohu and mrigal observed over a period of 12 days is presented in table 1. One hundred percent mortality was observed in catla by day 9, followed by rohu on day 10 and mrigal by day 11. No mortality was observed in the control group of fish injected with APW, during the observation period of 12 days. In all the three species of Indian major carp fingerlings, at the time of morbidity or death, 100% of the fish had severe swollen hemorrhagic areas. Tissue pathology of all the moribund Indian major carps was typical of a disease condition (Figs.1-10). Both injected (Figs. 1, 4 and 7) and non-injected (Figs. 5 and 9) sides and internal organs (Fig. 3) were extensively occupied by the mycotic lesions and there was severe myonecrosis (Figs. 6 and 10) in large areas of myotome and there was a massive proliferation of hyphae (Figs. 1, 2, 3, 7 and 8). These extensive pathological changes were always associated with severe gross lesions and morbidity or mortality. All fish (irrespective of species) sampled with this gross lesion had similar histopathological features.

Table 1. Cumulative mortality percentage of fingerlings of three different species of Indian Major Carps (catla, rohu and mrigal) injected with zoospores of *A. invadans* over a period of 12 days.

| Days post challenge | Cumulative percentage mortality of fingerlings of three different species of Indian major carps | | | |
|---|---|---|---|---|
| | Catla | Rohu | Mrigal | Control (injected with autoclaved pond water) |
| 4 | | | | |
| 5 | 0 | | | |
| 6 | 20 | | | |
| 7 | 60 | 0 | | |
| 8 | 80 | 20 | 0 | |
| 9 | 100 | 80 | 40 | |
| 10 | 100 | 100 | 80 | |
| 11 | 100 | 100 | 100 | |
| 12 | 100 | 100 | 100 | 0 |

For EUS to develop, the injected zoospores of *A. invadans* must be able to germinate in the muscle tissue. After germination, the hyphae must be able to proliferate and spread to the neighboring tissues and cause extensive necrotic pathology. The present study provides evidence that the injected zoospores of *A. invadans* were able to germinate in the muscle tissue of all the three species of Indian major carps and with time course,

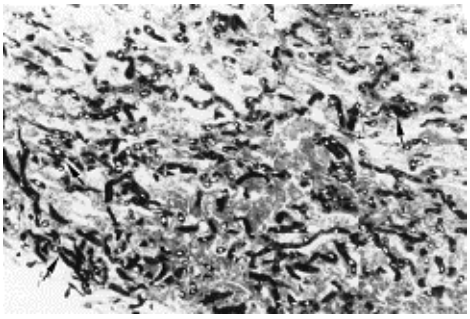

Figure 1. Mycotic lesion area of the injected side of catla at 6 days of post injection (dpi) showing massive proliferation of fungal hyphae (arrows) (Grocott – H&E, x100).

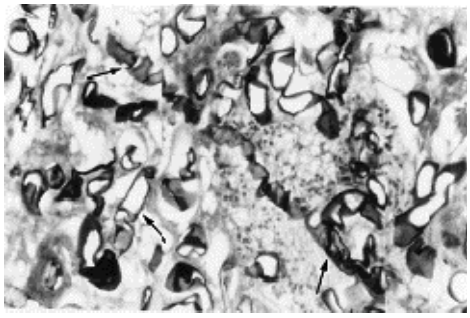

Figure 2. Higher magnification of the mycotic lesion area of catla at 6 dpi showing a large number of fungal hyphae (arrows) (Grocott – H&E, x400).

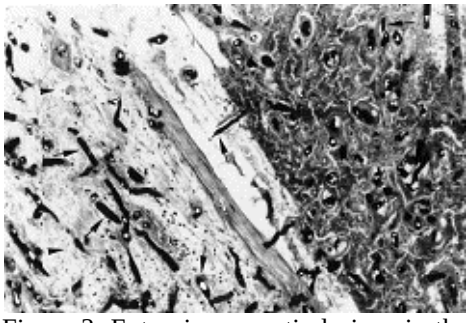

Figure 3. Extensive mycotic lesions in the myotome and internal organ (kidney) of catla at 6 dpi showing extensive myonecrosis (arrow heads) and fungal hyphae (arrows) (Grocott – H&E, x100).

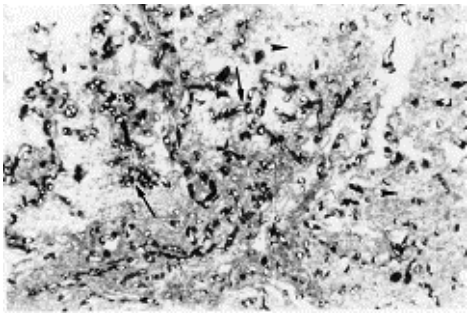

Figure 4. Mycotic lesion area in the injected side of rohu at 8 dpi showing extensive myonecrosis (arrow heads) and large number of fungal hyphae (Grocott – H&E, x100).

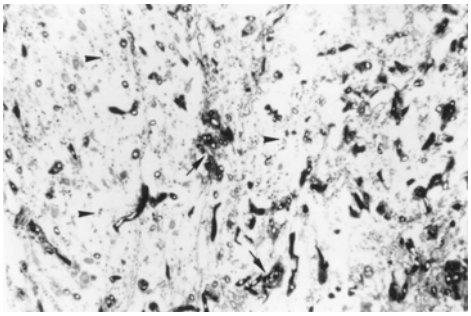

Figure 5. Mycotic lesion area of the non-injected side of rohu showing myonecrosis (arrow heads) and fungal hyphae (arrows) at 8 dpi (Grocott - H&E, x100).

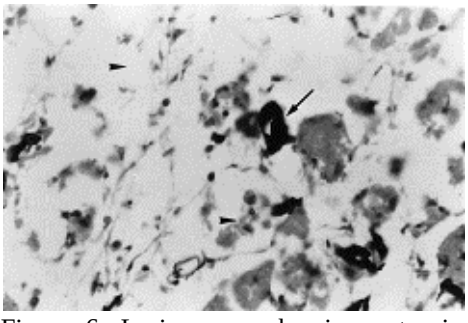

Figure 6. Lesion area showing extensive myonecrosis (arrow heads) and fungal hyphae (arrows) in rohu at 8dpi (H&E, x400).

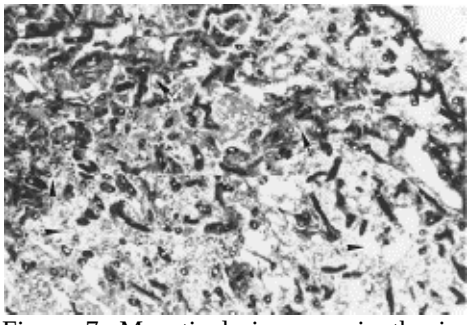

Figure 7. Mycotic lesion area in the injected side of mrigal at 9 dpi, showing massive proliferation of fungal hyphae (arrows) and myonecrosis (arrow heads) (Grocott –H&E, x100).

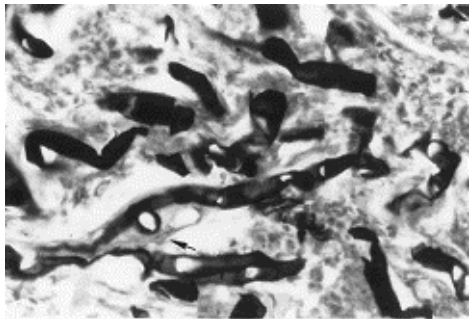

Figure 8. Higher magnification of the centre of mycotic lesion area of mrigal at 9 dpi showing a large number of fungal hyphae (arrow) (Grocott – H&E, x400).

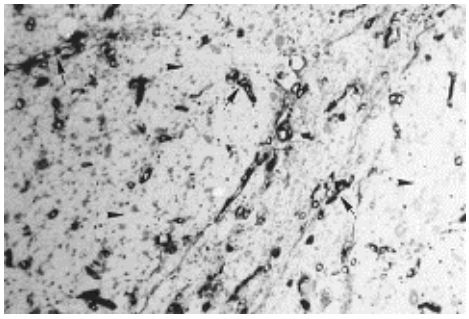

Figure 9. Mycotic lesion area of the non-injected side of mrigal at 9 dpi showing myonecrosis (arrow heads) and fungal hyphae (arrows) (Grocott - H&E, x100).

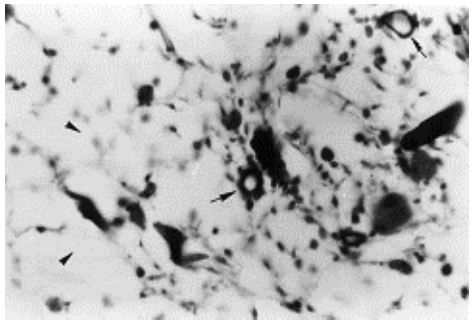

Figure 10. Lesion area showing extensive myonecrosis (arrow heads) and fungal hyphae (arrows) of mrigal at 9 dpi (Grocott – H&E, x400).

the germinated hyphae were able to proliferate massively and spread to neighboring tissues and caused extensive myonecrosis. Therefore, it was confirmed that, the mortalities observed were due to the germination and proliferation of the injected *A. invadans* zoospores.

The present experimental infection studies were undertaken in one of the southern states of India (Karnataka) and mortalities were recorded consistently between 6 and 11 days after injection with *A. invadans* zoospores. Mortalities due to artificial infection with *A. invadans*, in the case of Indian major carps have not been previously reported. However, in natural outbreaks, mass mortalities have been reported in infected farm ponds within 48 hours of introduction from the hatchery of fingerlings of Indian major carps (Chinabut and Roberts 1999). Interestingly, in natural outbreaks of EUS in several Southern Indian states (Jayaraman 1991; Vishwanath et al. 1997a; 1997b; 1998) Indian major carps present in many

water bodies have been found not to be affected. Therefore, epidemiological investigations in natural populations may help in shedding more light on the differential susceptibility of Indian major carps. However, based on the mortality pattern, gross pathology and tissue pathology of the present disease susceptibility experiment, it is confirmed that fingerlings of Indian major carps are highly susceptible to *A. invadans* infection and during the EUS season, the cultured populations of Indian major carps which are in these age groups are likely to be at high risk.

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