

EPIZOOTIC ULCERATIVE SYNDROME ON FRESH WATER AND BRACKISH WATER FISHES

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Natural transmission of invasive fungus is associated with Epizootic Ulcerative Syndrome (EUS) in Indian fresh and brackish water fishes. The fungus invades and proliferates in tissues away from the site of dermal ulcers. Totally five fresh water fishes and one brackish water fish species were selected for this study, and 100 individual of the each species were investigated for EUS infection, more than 10 fishes were infected with EUS positive from each species were identified by lesion on the fish tissue and it conformed by histopathology studies. The infection was occurred only during monsoon and post monsoon season not in summer and pre monsoon. Hyphae were associated with cellular debris and severe mycotic granulomas in muscle lesion. Finally concluded it the temperature variation is main reason for (EUS) infection and also it may due do caused by mixing of polluted water from domestic waste, fecal matter and Sewage through rain runoff.

Key words: Epizootic Ulcerative Syndrome, granulomas, freshwater fishes, *A. invadans*

Epizootic ulcerative syndrome (EUS) is a disease affecting a wide range of wild and farmed freshwater and estuarine fish. Pathological surveys of skin ulcers revealed that most contained oomycetes (filamentous protists that physically resemble fungi) (Blazer *et al.*, 2000; Law 2001; Noga *et al.*, 1988). EUS is currently defined as a seasonal epizootic condition of freshwater and estuarine warm water fishes of complex infectious etiology characterized by the presence of invasive *Aphanomyces* sp. Histologically, it is well established that the invading fungus causes significant necrotic changes in the skin and muscle tissue,

produces granulomas and ultimately results in the formation of dermal ulcers (Roberts *et al.*, 1993; Chinabut *et al.*, 1995). The ability of the fungus to penetrate fish tissues has even led to it being named *Aphanomyces invadans* (Willoughby *et al.*, 1995). The microbe associated with EUS is the oomycete, *Aphanomyces invadans* (Lilley and Roberts, 1997). Other diseases, e.g. red spot disease (RSD) in Australia (Callinan *et al.*, 1989), mycotic granulomatosis (MG) in Japan (Egusa and Masuda, 1971) and ulcerative mycosis (UM) in the USA (Dykstra *et al.* 1986), are now recognized as the same disease (Lilley and Roberts, 1997; Blazer *et al.*, 2002). The early stages of the disease are petechial hemorrhagic lesions of the skin that continue to develop into deep necrotic ulcers (Chinabut 1998).

Presumptive diagnosis of EUS can be based on clinical signs or demonstration of the EUS-characteristic mycotic granulomas. Identification of the primarily responsible pathogen, *A. invadans*, is essential for definitive diagnosis (Chinabut and Roberts 1999; OIE, 2006). Ulcerative skin lesions are frequently colonised by opportunistic water moulds (Blazer *et al.*, 1999) including other *Aphanomyces* spp. (Roberts *et al.*, 1993). They are likely to overgrow *A. invadans*, due to its slow growth rate compared to other species. Furthermore, bacteria can inhibit growth of *A. invadans*. PCR based approaches are extremely useful as diagnostic methods, where conventional methods of pathogen identification fail (Oidtmann *et al.*, 2004, 2006). 24 countries within 4 continents, Northern America, Southern Africa, Asia and Australia, have been recorded the present of the EUS (FAO, 2009). The disease with severe ulceration and causing heavy mortality of fishes from

small and large water bodies has been a major concern almost all over the countries of the Asia Pacific region since 1972. So far no fish disease has been as virulent and menacing as EUS in fish (Chinabut, 1994). Hence the present focused on the EUS infection on fresh water and brackish water fishes.

MATERIAL AND METHODS

Sample collection

Fish were collected from pond, canal and river in cuddalore, Tamilnadu, southeast coast of India, during Oct 2011 to Sep 2012. It is a common species occurring during all season. The fish were identified and examined for EUS infection.

Histopathology study

The infected fish wound tissue cut with small pieces and were fixed in 10% neutral buffered formaldehyde solution for 24 h. Histopathological observations were carried out following the method described by Coolidge and Howard (1979). After several wash in tap water the tissues were dehydrated through different grades of alcohol, cleaned in xylene and embedded in paraffin wax. Thin section of 3 μ m was cut with rotary microtome. The section were stained with Harris Haemotoxylin and counter stained with Eosin. The stained tissue was examined under microscope. Digital images of histological features were obtained using Lucia screen measurement system.

RESULTS

In the present study totally 100 individual from each species were collected in all seasons and examined for EUS infection, of these 43 individual were affected in fresh water fishes and 7 individual in brackish water fishes were affected by EUS during monsoon season, 10 individual in fresh water fishes and one brackish water fish were affected on post monsoon season, infection was not occurred on summer and pre monsoon season shown in (Table 1), which was observed different body part in the different fish (Fig 1) and also observed the different body part in one species of *Mugil cephalus* (Fig 2) were confirmed as

EUS-positive, based on the presence of mycotic granulomas in infected fish tissues.

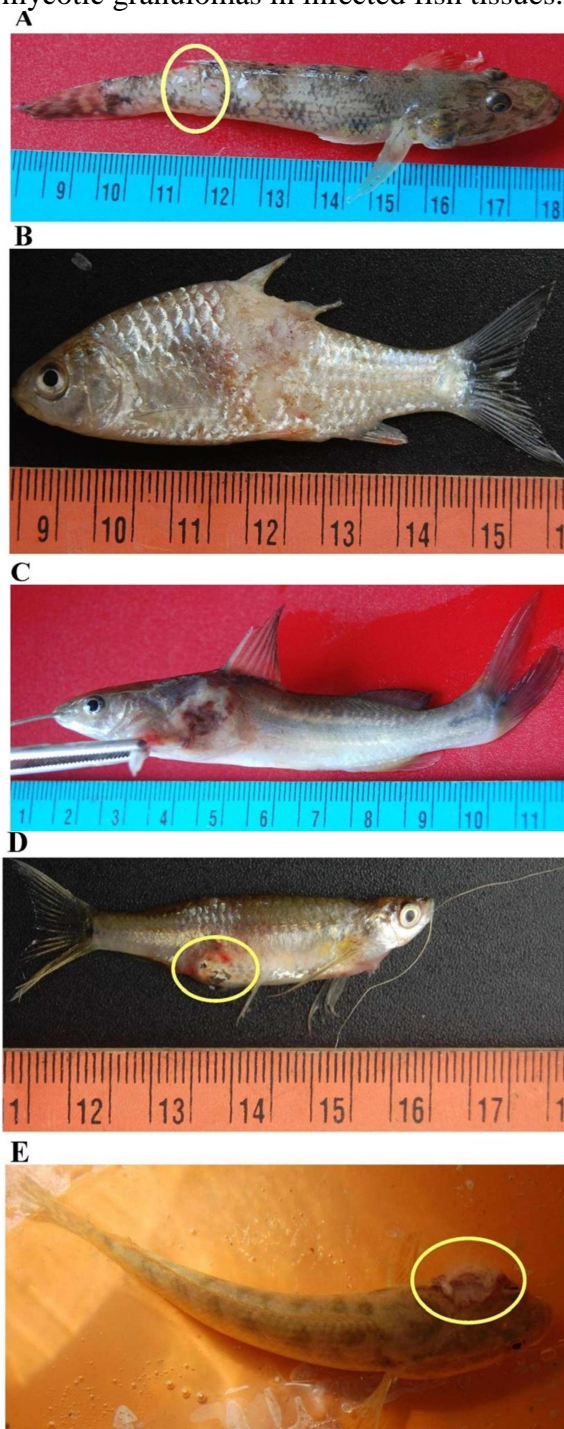


Fig-1 Photographs of wild fish species showing typical EUS lesions inn *Glossogobius* sp (A), *Puntius* sp (B), *Mystus* sp (C), *Esomus* sp (D), *Channa* sp (E)

The pathology in the form of myonecrosis, macrophage infiltration and mycotic granulomas was always severe in the area of the ulcer. Sections taken through the margins of ulcers showed fungal granulomas beneath intact epithelium. Surprisingly, more numbers of mycotic granulomas were

Table. 1. Prevalence of EUS infection in fresh water brackish water fish during October 2011 to September 2012

Fish species	Number of fish examined	Number of infected fish (%)			
		Monsoon	Post monsoon	Summer	Pre monsoon
<i>Glossogobius</i> sp	100	8 (8)	2 (2)	0 (0)	
<i>Puntius</i> sp	100	10 (10)	3 (3)	0 (0)	0 (0)
<i>Mystus</i> sp	100	5 (5)	1 (1)	0 (0)	0 (0)
<i>Esomus</i> sp	100	10 (10)	2 (2)	0 (0)	0 (0)
<i>Channa</i> sp	100	10 (10)	2 (2)	0 (0)	0 (0)
<i>Mugil cephalus</i>	100	7 (7)	1 (1)	0 (0)	0 (0)

also seen in the side of fish ulcer The fungal hyphae were found to invade in all directions from the centre of dermal ulcers (Fig 3).

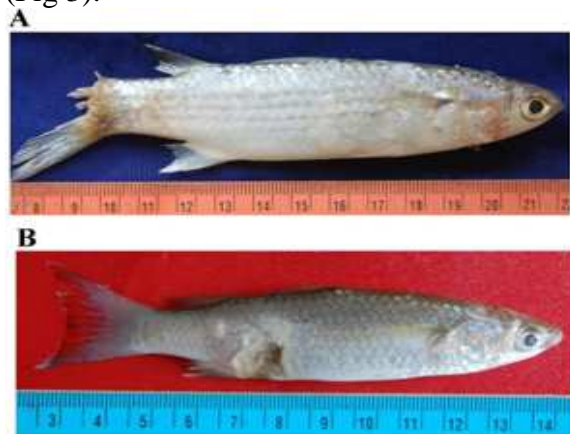


Fig.2. EUS infection in *Mugil cephalus*. Caudal fin (A), abdomen (deep up to viscera) (B).

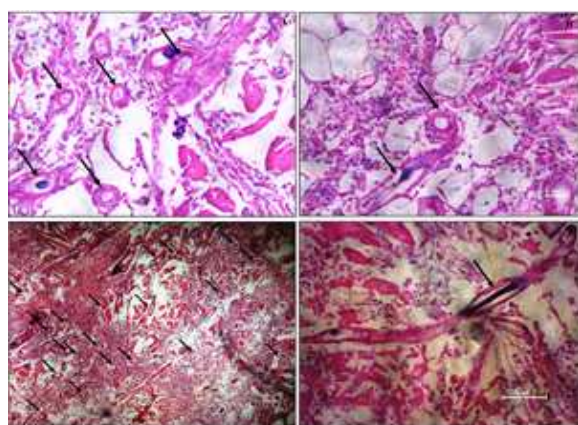


Fig. 3. Mycotic granulomas (A) Granuloma and fungal hyphae (B) more small granulomas (C) clear fungal hyphae (D) in EUS infected tissue.

DISCUSSION

The epizootic ulcerative syndrome (EUS) is a serious disease that affects many wild and

cultured freshwater fishes in the Philippines and in other Asian countries (Lilley *et al.*, 1998). The *Aphanomyces* zoospores can be horizontally transmitted from one fish to fish through the water. It is believed that only the zoospores are capable of attaching to the damaged skin of fish and germinating into hyphae. If the zoospores cannot find the susceptible species or encounter unfavorable conditions, they can form secondary zoospores. The secondary zoospores can encyst in the water or pond environment waiting for conditions that favor the activation of the spores. How the *Aphanomyces* pathogen or its spores survive after the outbreak is still unclear as outbreaks usually occur about the same time every year in endemic areas. There is no information to indicate that fish can be lifelong carriers of *A. invadans*. Generally, most infected fish die during an outbreak.

All the available evidence from the Asia-Pacific region conclusively suggests that a pathogenic, slow growing fungus of the *Aphanomyces* group is very closely associated with EUS (Willoughby *et al.*, 1995; Lilley *et al.*, 1997). Histopathological evidence revealed that the fungus associated with EUS of the Indian fishes is highly invasive in nature. The fungus is capable of invading tissues in all possible directions from the site of ulcer, presumably the point of initial entry. The presence of grossly visible lesions was usually combined with the presence of mycotic granulomas (Oidtmann *et al.*, 2008). Showed similar development of mycotic granulomas (Catap and Munday, 2002). Only relatively few reports have been confirmed by

demonstrating the presence of mycotic granulomas in histological sections (Songe *et al.*, 2011). The presence of fungal hyphae deep in the skeletal musculature and well away from the site of ulcers indicates the ability of viable fungal tips to proliferate rapidly and progress without getting walled off by the macrophages involved (Vishwanath *et al.*, 1998). Hyphae penetrated into dermal layers, subcutaneous tissues, and muscular layers (Takuma *et al.*, 2010).

In the present observation revealed that the EUS occurred during monsoon and post monsoon season, no infection was observed summer and pre monsoon season in fresh and brackish water fishes. It may due do mixing of polluted water from domestic vast, fecal mater and Sewage through rain runoff, it is similar with there is increasing evidence that the epizootics in freshwater are normally seen during cooler months and in brackish water during the monsoon (Chinabut *et al.*, 1995; Vishwanath *et al.*, 1997a). These trends were similar with some previous report Epizootic Ulcerative Syndrome associated with a fungal pathogen, in Indian fishes (Vishwanath *et al* 1998)

CONCLUSION

From the study, EUS were confirmed based on the presence of mycotic granulomas in infected fish tissues. More fishes were affected by EUS in monsoon season, low level in post monsoon season and were not occurred in summer and pre monsoon season so the *A. invadance* will grow based on the temperature variation.

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