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Mycotic Granulomatosis and Seasonality are the Consistent Features of Epizootic Ulcerative Syndrome (EUS) in Fresh and Brackishwater Fish of Karnataka, India

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Abstract

Histopathological investigations carried out between 1992 and 1994 on a large number of fresh and brackishwater fish of Karnataka, India, have confirmed that the ulcerative syndrome sweeping through Karnataka since 1991 is the devastating epizootic ulcerative syndrome (EUS). The two key diagnostic features of EUS, namely, oomycete involvement and proliferative mycotic granulomatosis, have been consistently demonstrated in all af-fected fresh and brackishwater fishes of Karnataka. Interestingly, Indian major carps and tilapia present in these affected water bodies were refractory to EUS. Mycotic granulomatosis and seasonality have been identified as the key features of this syndrome.

Introduction

It is now well established that a fungus is closely associated with epizootic ulcerative syndrome (EUS) in both fresh and brackishwater fish in the Asia-Pacific region (Fraser et al. 1992; Roberts et al. 1993; Mohan and Shankar 1995). The fungus is believed to be largely responsible, although not primarily in its own right, for the massive tissue damage and resulting skin ulcers on the fish body. Fungal presence and mycotic granulomatosis are recognized as the key diagnostic features of EUS (Lilley et al. 1992; Roberts et al. 1993). Because of the consistent association of fungus with EUS, the disease was defined as "a seasonal epizootic condition of fresh water and estuarine warm water fish of complex aetiology characterised by the presence of invasive *Aphanomyces* infection and necrotising ulcerative lesions typically leading to a granulomatous response" in the recent Overseas Development Agency (ODA) regional seminar on EUS held in Bangkok, Thailand (Roberts et al. 1994).

EUS is believed to have entered India in 1988, and since then has been causing large-scale mortalities in both freshwater and brackishwater fish (Das and Das 1993). Information available in India is largely confined to reports of prevalence of EUS and associated mortality. The diagnosis of EUS in the majority of these reports was based on the presence of gross clinical ulcers. Etiological investigations have so far been confined to the isolation and identification of bacteria (Pal and Pradhan 1990; Ali and Tamuli 1991; Pradhan et al. 1991). Little attention has been given to the differential diagnosis of ulcerative conditions and this has led to confusion regarding the range of species susceptible to EUS and its etiology. Even though the disease has been present in India since 1988, only the report of Mohan and Shankar (1994) on the presence of fungus and mycotic granulomas in skin and muscle tissue of EUS-affected *Puntius, Ophicephalus* and *Mugil* of Karnataka, India, conforms to the diagnostic features of EUS.

Establishing and confirming the diagnostic features of a disease are essential to its effective management. This paper presents histological evidence conforming with the latest definition and diagnostic features of EUS from large numbers of fresh and brackishwater fish of Karnataka, India. Evidence is also provided for the seasonal nature of EUS outbreaks.

Materials and Methods

About 200 ulcerated fish were collected from various fresh and brackishwater bodies of Karnataka from 1992 to 1994. Skin and underlying musculature were dissected from freshly killed individuals, and fixed in 10% neutral buffered formalin. Fixed tissues were processed using an automatic tissue processor, embedded in paraffin, and sections cut at 5-6 μm thickness and stained with hematoxylin-eosin following standard histological procedures (Roberts 1989). Grocott's methenamine silver staining (GMS) technique was used to demonstrate the presence of fungal hyphae.

Results and Discussion

Table 1 gives the range of species affected, period of outbreak and histological features of ulcerative lesions. A wide range of species were affected in both fresh and brackishwater ecosystems. In freshwater, a total of eight species were consistently affected. *Ophicephalus* was always severely affected, followed by *Puntius*. Indian major carps (*Catla catla, Labeo rohita, Cirrhina mrigala*) and tilapia (*Oreochromis*) stocked in many of these freshwater systems were not affected. In estuaries, seven species were affected, with bottom-feeding mullets (*Mugil, Valamugil*) being most susceptible. The syndrome was seasonal; in estuaries and brackishwater ponds, it invariably occurred following heavy rainfall and associated floods, when salinity dropped to less than 1 ppt (Mohan,

Table 1. Epizootiological and histological details of epizootic ulcerative syndrome in fresh and brackishwater fish of Karnataka, India.

Type of water body	Season	Species affected	Histological features		
			Necrotizing lesions on body	Presence of fungal hyphae	Fungal granuloma
Major and minor irrigation tanks	Aug. 1992	*Puntius* sp.	+	+	+
		Ophicephalus sp.	+	+	+
Rivers	Feb. 1993	*Puntius* sp.	+	+	+
Ponds	March 1993	*Ophicephalus* sp.	+	+	+
Estuaries	July-Sept. 1993	*Mugil* sp.	+	+	+
		Sillago sp.	+	+	+
		Scatophagus sp.	+	+	+
		Terapon sp.	+	+	+
		Etroplus sp.	+	+	+
		Platycephalus sp.	+	ND	ND
		Valamugil sp.	+	+	+
Open wells	Nov. 1993	*Ophicephalus* sp.	+	+	+
Minor and major irrigation tanks	Dec. 1993	*Ophicephalus* sp.	+	ND	ND
		Mystus sp.	+	ND	ND
		Puntius sp.	+	+	+
		Heteropneustus fossilis	+	+	+
		Glossogobius sp.	+	+	+
Irrigation tanks	Dec. 1993 to Jan. 1994	*Ophicephalus* sp.	+	ND	ND
		Puntius sp.	+	+	+
		Mystus sp.	+	ND	ND
		Wallago sp.	+	ND	ND
Irrigation tanks	Feb. 1994	*Ophicephalus* sp.	+	+	+
		Puntius sp.	+	+	+
		Esomus sp.	+	+	+
		Puntius sp.	+	+	+
Estuaries	July-Aug. 1994	*Mugil* sp.	+	+	+
		Sillago sp.	+	+	+

+ = Present, ND = Not done

unpubl. data). In freshwater, the syndrome was predominantly seen during cooler months of the year (November-January). During the study period, the syndrome did not reappear in freshwater systems where it had previously occurred. Interestingly, it reappeared in estuarine systems, but the prevalence and severity were much lower than previously observed.

Sections taken through the ulcers revealed the presence of massive numbers of highly invasive, broad, non-septate fungi. Fungal invasion in all the fresh and brackishwater species examined was associated with inflammatory changes, macrophage response, massive dermatitis, myofibrillar necrosis and proliferative granulomatous response (Figs. 1-3). The fungal-induced pathological changes were similar in all the species. However, there were noticeable differences in the nature of inflammatory response between the species. In some

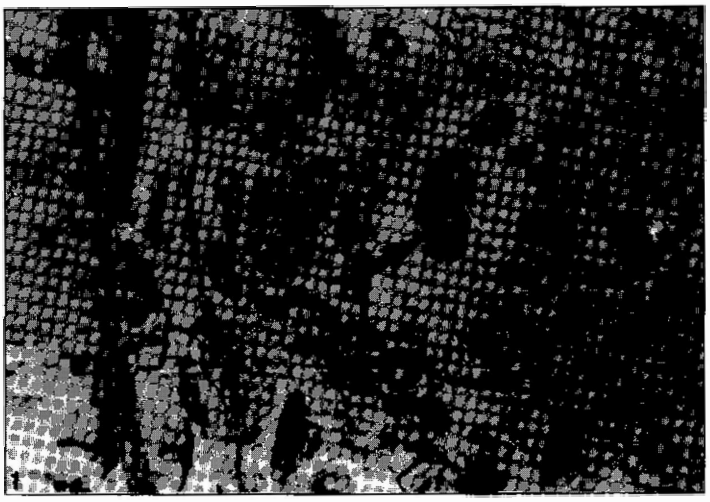

Fig. 1. *Esomus* sp. (GMS - HE, x150)

Fig. 2. *Glossogobius* sp. (H&E, x150)

Fig. 3. *Etroplus* sp. (H&E, x150)

Fungal hyphae (arrow head) within the granulomas (arrow) in the necrotizing lesion of skin and skeletal musculature.

species such as *Glossogobius*, there was fibrotic fusion of adjacent granulomas resulting in the formation of coalesced granulomas (Fig. 2). The centers of such advanced granulomas had apparently degenerate fungal hyphae and necrotic muscle fibers. In species like *Etroplus* (Fig. 3) and *Sillago*, the granulomas were not several layers thick, unlike the typical thick and mature granulomas found in severely affected species like *Ophicephalus, Mugil, Puntius* and *Glossogobius* (Fig. 2).

The epizootiological and histopathological findings of the present study (Table 1) strongly agree with the definition and diagnostic features of EUS as proposed in the recent ODA regional seminar on EUS (Roberts et al. 1994). Histopathological results clearly and consistently show that a specific type of broad, non-septate fungus was involved in all affected fish species. The host response was characteristic and consistently present in a wide range of fish species. Chronic inflammatory tissue surrounding the fungal hyphae to form massive epitheloid granulomas was particularly evident in all the specimens studied. Similar observations have been made in a range of EUS-affected species in Southeast Asia (Roberts et al. 1993).

There are several reports from Northern India of Indian major carps being susceptible to EUS, but surprisingly the diagnostic features have not been demonstrated so far. In contrast, in Bangladesh, the diagnostic features of EUS have been demonstrated in Indian major carps (Roberts et al. 1993). In the present study, Indian major carps and tilapia stocked in many of the affected freshwater systems were refractory to EUS.

Mycotic granulomatosis, a characteristic feature of EUS, is also a consistent feature in *Mugil cephalus* with red spot disease in Australia (Callinan et al. 1989) and ayu (*Plecoglossus altivelis*) with mycotic granulomatosis in Japan (Hatai et al. 1977). In all three cases, the fungus involved has been identified as *Aphanomyces* (Hatai et al. 1977; Fraser et al. 1992; Roberts et al. 1993). During the study period, *Aphanomyces* was isolated from *Ophicephalus* and *Mugil* (Mohan, unpubl. data). The similarity of these diseases in chronologically and geographically isolated cases suggests a possible common cause and indicates that the fungus is more important than previously recognized.

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