Epizootic Ulcerative Syndrome: Information up to 1997

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Epizootic ulcerative syndrome or EUS has been endemic in many countries in Asia and the Indo Pacific region since 1980. It has affected a wide range of fish species. Infected fish reveal dermal ulcerative lesions throughout the body. The outbreaks occur at certain times of the year, normally after flooding followed by cool weather. Typical clinical signs of the early stages of this disease are the petechial haemorrhagic lesions on the skin which later develop into deep ulcerative lesions throughout the body. Mycotic granulomas spread throughout the lesions and also into some of the internal organs. These histopathological observations are key characteristics in the identification and confirmation of outbreaks of EUS. The epidemiology of this disease is still uncertain, however, a similar disease called mycotic granulomatosis was reported in Japan in 1971. In the following year red spot disease was reported in estuarine fish from Queensland, Australia. Since then the disease has spread to almost all countries in Southeast Asia and the Indian subcontinent. Recently (1996) an EUS outbreak in Pakistan has been reported. It seems clear from the epidemiological information, and research carried out, that EUS is infectious in nature. Parasites, bacteria and viruses found in diseased fish are considered to be the secondary agents. Aphanomyces invadans, a pathogenic water borne fungus, most likely plays an important role in these disease outbreaks.

Key words: EUS, epizootic ulcerative syndrome, Aphanomyces invadans, mycotic granulomatosis, red spot disease

An outbreak of an unknown ulcerative infection in various species of fish in Thailand in 1981 drew the attention of a number of scientists to work together and study on the aetiology. FAO played an important role in supporting a detailed survey and the British Department for International Development (DFID) contributed significant funding for research into the cause of the disease. Epizootic ulcerative syndrome or EUS is the name given to this disease since 1986 (FAO, 1986). The definition of EUS is “a seasonal epizootic condition of freshwater and estuarine warm water fish of complex infectious aetiology characterised by the presence of invasive Aphanomyces infection and necrotising ulcerative lesions typically leading to a granulomatous response” (ODA, 1994). It is now admitted that red spot disease (RSD) in Australia (Callinan et al., 1995) and mycotic granulomatosis (MG) in Japan (Miyazaki and Egusa, 1972) correspond to EUS. The continuous spread of EUS from one country to the other for nearly three decades confirms that this is an international problem and causes significant economic loss.

Epizootic

The disease called mycotic granulomatosis was first found in pond raised goldfish Carassius auratus (Miyazaki and Egusa, 1972), and ayu Plecoglossus altivelis in various regions in Japan in 1971 (Miyazaki and Egusa, 1973a). Wild species such as formosan snakehead Channa maculata, Japanese trident goby Tridentiger obscurus and black mullet Mugil cephalus were also affected by the disease at this time (Miyazaki and Egusa, 1973b, c). Later, the causative agent of mycotic granulomatosis was identified as Aphanomyces piscicida sp. nov. by Hatai (1980). In the following year, 1972, red spot disease (RSD) was first reported in estuarine fish from the Burnett River, Queensland, Australia (Rodgers and Burke, 1981). In the following year, 1972, red spot disease (RSD) was first reported in estuarine fish from the Burnett River, Queensland, Australia (Rodgers and Burke, 1981). Lately, outbreaks of RSD have been reported in many species of estuarine and freshwater fish in New South Wales (Callinan et al., 1989), North Territory (Humphrey and Langdon, 1986; Pearce, 1990) and Western Australia (D. Pass,
unpublished). Sea mullet *Mugil cephalus*, sand whiting *Sillago ciliata*, yellow fin bream *Acanthopagrus australis* and barramundi *Lates calcarifer* were some of the economically important estuarine species affected. Outbreaks of red spot disease have occurred in farmed silver perch *Bidyanus bidyanus*, which is a freshwater species of Southern Australia (Rodgers and Burke, 1981).

Since 1971–72 the disease gradually spread to many countries in Asia-Pacific region (Fig. 1). Catfish cultured in Mekong Delta, Vietnam was reported to be infected by EUS in 1973 (ODA, 1994). Unfortunately, this information was not supported by the histopathological confirmation. In 1975, there was a report on EUS outbreak of some freshwater fish in Papua New Guinea. By the year 1980 it spread to Malaysia and Thailand.

![Map of countries affected by epizootic ulcerative syndrome.](image-url)
In 1982–3, a severe EUS outbreak in Thailand affecting snakehead *Channa striata*, *Puntius* sp. and many species of rice-field fish was recorded (Tonguthai, 1985). The outbreak was also reported in East Kalimantan, Indonesia in 1982 and 1984, causing serious mortality on wild species such as snakehead, catfish, sand goby *Puntius* sp. and kissing gouramy in natural water resources (ODA, 1994). Cambodia, Laos and Myanmar were suffering by EUS at the same time in 1984. The first confirmed EUS outbreak of some freshwater fish in Laguna de Bay, the Philippines was recorded in 1985–86. As early as 1987, *Puntius* sp. and snakehead in the western Province of Sri Lanka were infected by EUS. After the severe flooding in 1988, the first EUS outbreak was reported in Bangladesh. Major carp seem to be the main affected species. In the same year, EUS occurred in North East India and spread throughout the whole country and also to Nepal in the following year (ODA, 1994). By 1996 it was confirmed that EUS had spread to the upper part of Indus River in Pakistan (Kanchanakhan, 1996).

**Pathogenic Agents**

Based on the investigation of the pathogenic organisms found from EUS-infected samples, a wide range of aetiologies were attributed to EUS, such as parasites, bacteria, viruses and fungi.

Several species of parasitic protozoan (*Chilodonella* sp., *Trichodina* spp., *Costia* sp., *Ichthyophthirius* sp., *Epistylis* sp. *Glossatella* sp. and *Scyphidia* sp.), myxosporean (*Thelohania* sp. and *Henneguya* sp.), monogenean, and crustacean (*Lernaea* sp.) were recorded from diseased fish (Callinan et al., 1997; Reungprach et al., 1983). Nevertheless, there was no proven evidence to confirm that these parasites are the causative agent of the disease. Parasitic infections possibly induce stress in fish thus predisposing them to infection (Subasinghe and Jayasinghe, 1990).

*Aeromonas hydrophila*, *A. sobria*, *Pseudomonas* spp., and *Vibrio* spp. were the species of bacteria isolated from the internal organs of EUS infected fish (Burke and Rodgers, 1981; Llobrera, and Gacutan, 1987; Tonguthai, 1985). Among these bacteria, *A. hydrophila* was the most commonly isolated species from the advanced stages of infected fish. It is usually not isolated from the early stage of the disease. On the other hand, *A. hydrophila* is known to be a normal microflora of fish and aquatic environments and recognised as an opportunistic pathogen.

A numbers of birnaviruses, rhabdoviruses and reoviruses have been isolated from diseased snakeheads and other susceptible species. Frerichs suggested that all of these viruses most probably represent adventitious infections unrelated to the outbreak of EUS because of the heterogeneous nature of these viruses, together with a very low and non-consistent level of recovery from epizootics of disease. He also concluded that the role of viruses in the aetiology of this disease remains unproven (ODA, 1994).

At least two species of fungi had been isolated from EUS infected fish, *Achlya* sp., which was isolated from the superficial area of the lesions (Pittchayangkula and Bodhalamik, 1983; Subasinghe et al., 1990) and *Aphanomyces* sp. that is normally isolated from the muscle area near the lesions (Egusa, 1992, Fraser et al., 1992; Hatai et al., 1994; Roberts et al., 1993). Recently, it has been shown that all *Aphanomyces* spp. identified from EUS samples not only are the same species but they also represent a single clonal lineage (Lilley et al., 1997). Therefore, it is confirmed that the fundamental aetiological agent of EUS is an oomycete fungus, *Aphanomyces invadensis* (Willoughby et al., 1995). However, the valid taxonomic name of this species according to the International Code of Botanical Nomenclature (ICBN) is *A. invadans* (Lilley et al., in press). This specific pathogenic fungus showed the ability to invade the skin lesions of EUS-infected fish. Isolation of this particular fungus is difficult as it is a slow growing strain and most of the hyphae in the muscle are dead except for the tips that are penetrating deeper into the muscle tissue (Roberts et al., 1993).

**Pathogenicity of the fungus**

After success in isolating the slow growing fungus, *A. invadans*, from EUS-infected fish in various countries (Egusa, 1992; Fraser et al., 1992; Willoughby et al., 1995), pathogenicity studies were conducted. The intramuscular injection of spore suspensions into some susceptible fish species (snakehead, barbs) demonstrated a severe necrotising myogranulomatous condition at the injected site similar to the pathological feature of fish naturally infected with EUS. At the later stages, the fungus invaded rapidly to the other internal organs causing death to the host. The diameter of the hyphae of fungus in lesions was always larger and the walls thicker than in cultured specimens (Roberts et al., 1993). The exposure of healthy susceptible fish to either suspension of spores or hyphae of *A. invadans* proved impos-
sible in inducing typical EUS, unless a skin abrasion was made. However, co-habitation transmission experiments with EUS-infected fish were successful (Balasuriya et al., 1990; Cruz-Lacierda and Shariff, 1995).

Histopathology

Lesions in all EUS-infected fish are similar, except that in a tough snakehead chronic lesions were progressed. This is because snakehead was surviving longer and thus the lesions had developed to a very advanced stage, where as the other species died before this stage was reached.

Grossly the lesions were generally small red erosions on the body, head and fins. In the advanced stage, deep haemorrhaged ulcers spread through the whole body of the infected fish. Fungal mycelium was commonly found on top of the ulcerative lesions. The internal organs of the early stage of infected fish showed only minimal inflammatory response. It is important to note that diseased fish with not too advanced lesions, kept in better water quality conditions, often recover and undergo a healing process leaving dark scars at the sites of infection.

Histopathological observation of the early stage lesions found acute spongiosis with loss of epithelial cells. Degenerative changes were observed in the dermis, with hyperaemia, haemorrhages and inflammatory cells infiltrating between the fibres of the dermis. Inflammatory exudate and haemorrhages were also found in the hypodermis. Sarcolysis with haemorrhages and inflammatory exudate were obvious in the more advanced stage. The fungal hyphae were enclosed by a well developed epithelioid cell layer. Mycotic granulation of these non-septate fungus hyphae spread through the infected muscle and other internal organs in the very late stages. In the advanced stage, there were no muscle fibres in the granuloma tissues. They were replaced by fibrosis, inflammatory cells and newly developing capillaries (Chinabut et al., 1995). This distinct feature of the typical mycotic granulomas in the lesions of EUS-infected fish provides important histopathological information which can be used to distinguish EUS from other fish ulcerative diseases.

Prevention and treatment

The outbreak of EUS in the wild stock is unlikely to be prevented or treated. However, in cultured systems a simple and effective form of prevention in an endemic area is to culture species which are resistant to EUS; such as Chinese or European carps, tilapia and milk fish. It is interesting to note that EUS outbreaks in estuarine fish have been recorded only in water with salinity lower than 2 ppt (Callinan et al., 1997).

In case that susceptible species such as snakehead, Puntius sp. etc. have to be farmed in an endemic area, good husbandry is essential; for example seed stock should be obtained from hatcheries which are recorded free from EUS outbreak, water supply should come from a well or bore, and disinfected equipment should be used on farms.

Success in pond treatments were reported by using agricultural lime at the amount of 150–600 kg/ha in 1 m deep ponds at 2–4 weeks intervals depending on the pH of the pond water (Lilley et al., 1997). However, addition of lime at 2 kg/100 m² during the culture period in pond trials failed to prevent EUS lesions induction in abraded African catfish (Callinan, unpublished).

Pond trials have shown that 5 ppm Coptrol (a chelated copper compound) prevented induction of EUS lesions in abraded African catfish while malachite green (0.1 mg/l) was partly effective and formalin (25 mg/l) was ineffective (Callinan, unpublished). Malachite green, hydrogen peroxide and proxitane 0510 showed a fungicidal activity against A. invadans in in vitro trial (Lilley and Inglis, 1997).

Conclusion

It should be emphasized that outbreaks of EUS have continued to occur periodically in all the target countries. The immunity of fish surviving EUS infections should be the subject of interest because the record from many countries showed that the severity of EUS outbreak after the first few years tends to decrease (Mohan and Shankar, 1994; Bondad-Reantaso et al., 1992). The serious implications for the development of vaccines against EUS should be deliberated because there was a report on the strong reaction between the sera from immunized fish with the spores and mycelium of pathogenic strains of Aphanomyces and the extracts of pathogenic strains of Aphanomyces (Thompson et al., 1997).

Pakistan is the latest country suffering from EUS outbreak, therefore, the study on epidemic and monitoring of this disease in Pakistan should be considered. The rapid diagnosis technique should be developed to distinguish EUS from other ulcerative diseases.
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