

Full Length Research Paper

Outbreak of epizootic ulcerative Syndrome (EUS) in *Seranochromis robustus* Fish species in Darwendale dam, Zimbabwe

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The aim of the study was to investigate the causes of mortality of fish in Darwendale Dam in Zimbabwe. Findings of laboratory gross examination of lesions were consistent EUS. Histopathology examination showed that the fish had mycotic granulomas. Fish of all ages were affected. High mortalities and severity of ulceration suggests a relatively recent invasion by *A. invadans* at Darwendale dam. EUS spread from Darwendale to other areas due to improper surveillance and poor control strategy due to lack of EUS knowledge by fishermen and authorities. More studies needed to be done in Zimbabwe to determine species of fish affected, strain *A invadans* circulating and control measures to prevent spread of diseases. EUS was confirmed in *Seranochromis robustus* in Zimbabwe in 2013. The study is the first published account of infection with *Alphanomyces invadans* in the wild fish populations (*Seranochromis robustus*) of the Darwendale Dam in Zimbabwe.

Key words: EUS, Darwendale, Zimbabwe, *Alphanomyces*, *Invadans*, wildfish.

INTRODUCTION

Infectious disease emergence and re-emergence are a major concern for medical, veterinary and conservation-related disciplines (Johnson and Paull, 2011). Epizootic ulcerative syndrome (EUS) is a fish disease of international significance and reportable to the Office International des Epizootics (Boys et al., 2012). The emergence and spread of aquatic freshwater diseases are a major conservation concern (Johnson and Paull, 2011). Zimbabwe had not experienced any significant and

documented outbreaks of fish disease in wild stocks until recently. Epizootic ulcerative syndrome (EUS) or 'red-spot' is an ulcerative syndrome of fish which affects a range of native species (Humphrey and Pearce, 2004). It begins as a small area of reddening over a single scale, which subsequently spreads to involve a number of adjacent scales; this is the characteristic 'red spot'. As the condition progresses, the 'red-spot' expands and deepens, giving a deep ulcer, which sometimes extends into the abdominal cavity (Humphrey and Pearce, 2004). A pathogenic fungus, *Aphanomyces invadans* (*A. invadans*) causes the disease EUS. Infection occurs when motile spores in the water are attracted to the damaged skin of fish. The spores penetrate the skin and germinate, forming fungal filaments or hyphae.

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These hyphae invade widely into the surrounding skin and deeply into underlying muscle tissues, resulting in extensive ulceration and destruction of tissues (Humphery and Pearce, 2004). The *A. invadans* oomycete needs predisposing factors that lead to skin damage, such as parasites, bacteria or virus infection or acid water, to initiate the clinical signs of the disease (Lilley and Roberts, 1997). The disease, EUS is a devastating seasonal epizootic condition of great importance in wild, farmed freshwater and estuarine fish (Songe et al., 2012). Due to EUS fish productivity in all susceptible fish species will drastically be reduced (Songe et al., 2012). However, EUS-affected fish often die because of bacterial septicaemia caused by pathogenic aeromonads entering the circulation through haemorrhagic dermal lesions that characterizes the disease (Das et al., 2009). EUS is a seasonal epizootic condition resulting in large hemorrhagic necrotizing ulcers typically producing a granulomatous response.

MATERIALS AND METHODS

The case study occurred in October 2012 in Darwedale dam, 40km from Harare, Zimbabwe. This particular outbreak was official recorded by the Wildlife Unit in the Department of Livestock and Veterinary Services. EUS was diagnosed using laboratory techniques and confirmed according to OIE manual in the microbiology laboratory, Faculty of Veterinary Science, University of Zambia.

Fish were caught using gill nets and inspections were done on fish catches directly from fishermen, catches and any fish showing clinical signs and apparently infected fish were prepared for examination. The fish were identified to species level following the procedure "species accounts" as described by (Coke, 2002). Whole fish samples were collected and cooled on ice in a cooler box and immediately transported to the Central Veterinary laboratory, Harare, Zimbabwe for further processing and shipment to University of Zambia for gross pathological examination and sampling for histology. Suspected fishes with EUS-like lesions were further examined in detail at the Central Veterinary laboratory for species identification and for pinpoint red hemorrhagic spots, localized swelling, and raised areas on the body surfaces, protruding scales, loss of scales, skin ulceration, and exposure of underlying tissues. Collections of samples were based on OIE procedure which states that in outbreak investigations, diseased fish with ulcerative lesions or red spots on the body should be sampled (OIE, 2013). At the Laboratory, skin and muscle samples exhibiting lesions were collected and fixed in 10% buffered formalin according to the OIE manual of diagnostic tests for aquatic animals (OIE 2013). The samples preserved in formalin were then processed and analyzed by histological processing, i.e., dehydration

through ascending alcohol grades, clearing in xylene, impregnation with wax, cutting at about 5 µm, mounting on a glass slide, complete de-waxing, and staining in haematoxylin and eosin (Oidtmann et al., 2013). The tissue sections were then examined under a light microscope. Staining also included the use of Grocott's modification of Gomori's methenamine silver, to demonstrate the definite presence of the fungus (Saylor et al. 2010). The stained tissue sections were then examined under a light microscope for mycotic granulomas. The clinical signs and gross pathology of the ulcerated fish, plus the presence of mycotic granuloma in affected tissues, indicated a positive diagnosis for *A. invadans*.

RESULTS

Fish younger than one year appeared to be the most seriously affected and sporadic cases were reported in mature fish of *S. robustus*.

A total of 1000 fishes *S. robustus*, Tilapia and catfish were investigated during the study period. Of these fishes, 10% were found with common lesions of skin ulceration and bloody patches on the body.

EUS is one of the aquatic diseases implicated in mass mortalities of cultured and wild fish in many countries. (Boys et al., 2012). Fish from infected waterways, especially those with lesions of EUS, should not be relocated to other waterways. In captive fish, early 'red-spot' lesions may respond to topical treatment with an antiseptic iodophore solution. Increasing salinity of holding waters may prevent outbreaks of EUS in aquaculture ponds (Humphery and Pearce, 2004). This study confirmed the presence of the oomycete in susceptible fish species (*S. robustus*) collected from Darwadale dam. More than 100 fish species have been reported to be affected by EUS worldwide (Baldock et al. 2005), but only relatively few reports have been confirmed by demonstrating the presence of mycotic granulomas in histological sections (Songe et al., 2012). Skin ablation is thought to facilitate attraction of and infection by zoospores of *A. invadans* (Songe et al., 2012) This could be attributed to the development of immunity against the disease especially in larger fish. In Zambia the study of of EUS concluded that, a number of fish species of economic value are affected with EUS in the Zambezi River, while only *Tilapia sparrmanii* (*T. sparrmanii*) was found to be resistant. The low winter temperature (20-25°C) is ideal for EUS outbreaks. EUS infection is waterborne. EUS had been reported in more than 100 fish species in both freshwater and estuarine environments and this makes it important to further study the epidemiology of EUS in Zimbabwe

Outbreaks of EUS have been reported in dams more than 200kms away from Darwendale dam after the initial cases. Studies done in Murray Darling Basin River System

in Australia found out that the epizootic reported covered a 200 km section of the Barwon-Darling River in the northern region of the Murray-Darling Basin. Infection with *A. invadans* has been reported from more than 20 countries on four continents. Movements of live ornamental fish from infection with *A. invadans*-infected countries might spread the disease as was the case with the outbreak in Sri Lanka (Balasuriya, 1994). Flooding also caused the spread of infection with *A. invadans* in Bangladesh and Pakistan (Lilley *et al.*, 1998). Once an outbreak occurs in rivers/canals, the disease can spread downstream as well as upstream where the susceptible fish species exist. Ensuring that water from infected rivers does not come into contact with fish culture ponds could prevent the spread of the disease. Although EUS is not a zoonotic, outbreaks of EUS has been associated with outbreaks of some of the bacteria e.g. *Aeromonas* which have a broad host range and have been implicated in the cause of human gastroenteritis and wound infections arising out of a direct contact with contaminated soil and water (Das *et al.*, 2009). There are no specific control measures in fish for EUS in natural environments. Little is known of EUS disease pathogenicity, distribution or susceptibility of species to infection in wild populations in river system of Zimbabwe. More studies are required to determine the susceptibility to EUS of all commercially important fish species in Zimbabwe.

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