UTF/077/ZAM: Technical Assistance to the Zambia Aquaculture Enterprise Development Project (ZAEDP)

Session 2: Introduction to Epizootic Ulcerative Syndrome

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Outline of lecture

Introduction

- Background
- Causative agent of EUS
- Effects of infection with EUS
- Global distribution of Disease

Pathogenesis of EUS

- Life Cycle of *A. invadans*
- Pathogenicity studies

Risk factors

Control

Summary

Background

- Epizootic ulcerative syndrome (EUS) is a severely infectious disease of fish
- Known to affect more than 100 types of wild, farmed freshwater and estuarine fish.
- World Organization for Animal Health (OIE): confirmed that EUS is a seasonal epidemic caused by infection with the highly invasive, deeply penetrating pathogenic oomycete *Aphanomyces invadans* or *A. piscicida*, and is listed among those diseases that must be notified
- Red Spot Disease, Mycotic Granulomatosis, Ulcerative Mycosis and Epizootic Granulomatous Aphanomycosis are all synonyms for EUS

Distribution

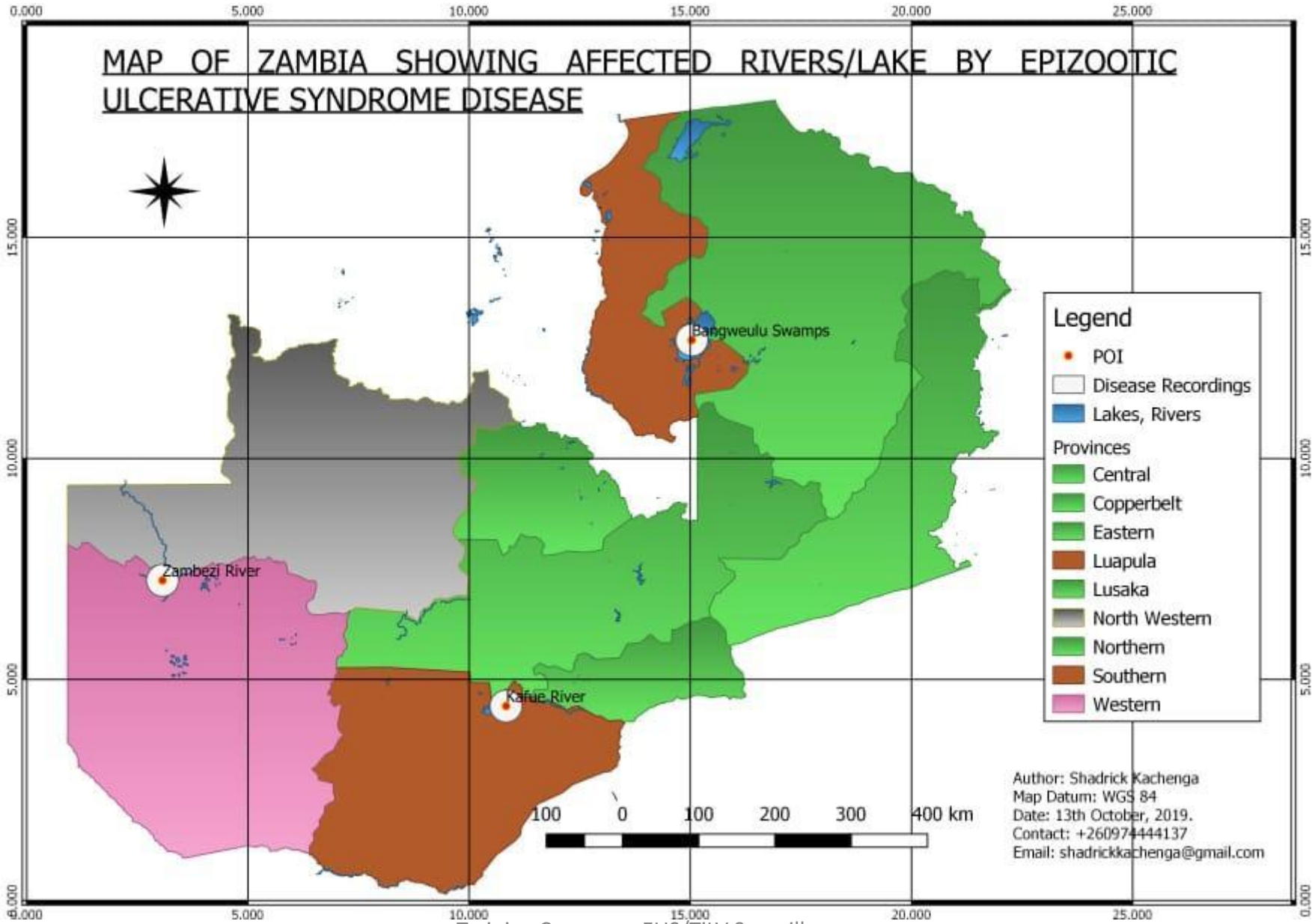
- First report of the disease: Japan, where it is known as mycotic granulomatosis in 1971
- In Australia (reported in 1972), where it primarily affected estuarine mullet, it was called Red Spot Disease (RSD)
- Since the early 1980s, EUS has spread westwards across the South-East and South Asia, affecting over 100 species of freshwater fish
- First reported in Bangladesh in 1988

Distribution

- First case in the USA: 1978. Outbreaks of Ulcerative disease in menhaden, *Brevoortia tyrannus*, were very similar to EUS in Asia
- The pattern of spread between and within countries was consistent with progressive dissemination of a single infectious agent. On the basis of findings from studies conducted by Lilley and others, the authors suggested that *A. invadans* achieved its colonisation of Australia, Asia and by implication USA, in one relatively rapid episode, consistent with reports of outbreak occurrence.

EUS in Africa and Zambia

- The first outbreak of EUS Zambia and other countries sharing the Zambezi River occurred in 2007. In Zambia EUS is endemic on the Zambezi River and its tributaries
- The disease has spread to other river systems such as Kafue. It has also recently been recorded in the Bangweulu swamps
  - The outbreak was of great significance as the Bangweulu swamps drain into the Congo River in neighbouring Democratic Republic of Congo, Africa's largest drainage system with an extensive and diverse fish fauna previously unaffected by EUS


MAP OF ZAMBIA SHOWING AFFECTED RIVERS/LAKE BY EPIZOOTIC ULCERATIVE SYNDROME DISEASE
Bangweulu Swamps
Zambezi River
Kafue River
Legend
POI
Disease Recordings
Lakes, Rivers
Provinces
Central
Copperbelt
Eastern
Luapula
Lusaka
North Western
Northern
Southern
Western
100 0 100 200 300 400 km
Author: Shadrick Kachenga
Map Datum: WGS 84
Date: 13th October, 2019.
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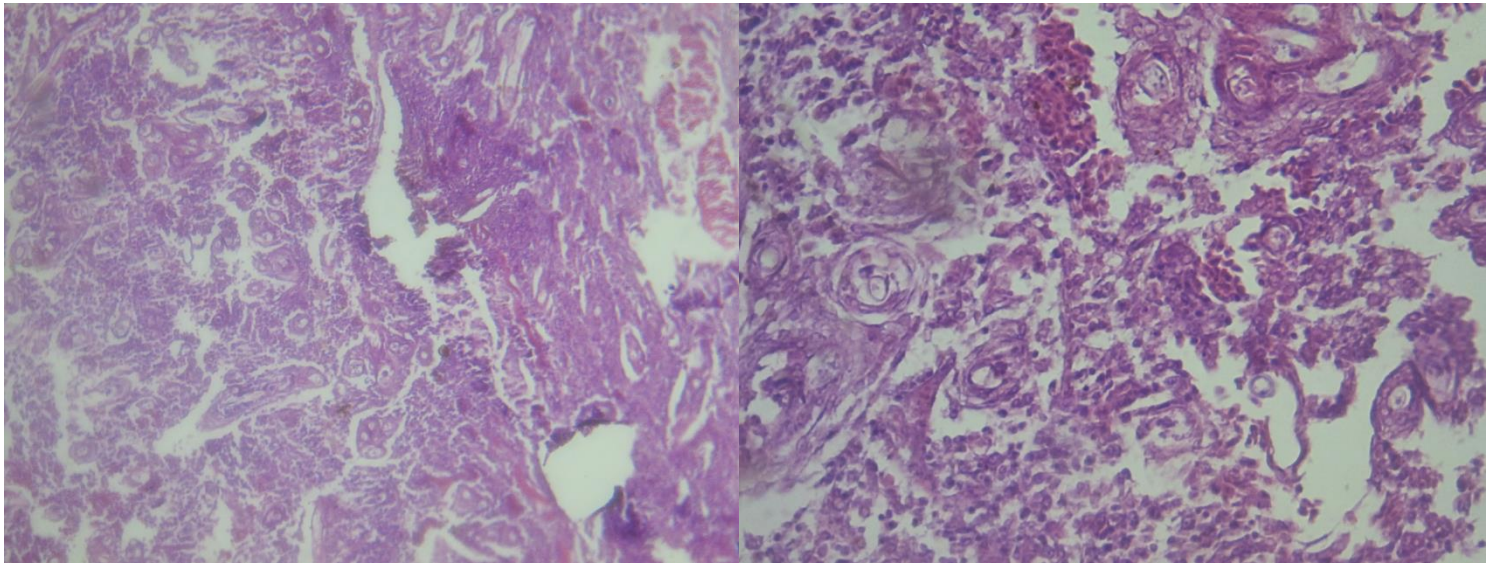
Pathogenesis

- *A. invadans* has been shown to be slow-growing and thermo-labile in culture
- The *A. invadans* zoospores can be horizontally transmitted from one fish to another through the water. It is believed that only the secondary zoospores or free-swimming stage zoospores are capable of attaching to the damaged skin of fish and germinating into hyphae
- In some cases prior damage to skin is necessary before lesions can be induced in fish

Pathogenesis

Presence of *A. invadans*: accompanied by an intense granulomatous inflammatory response



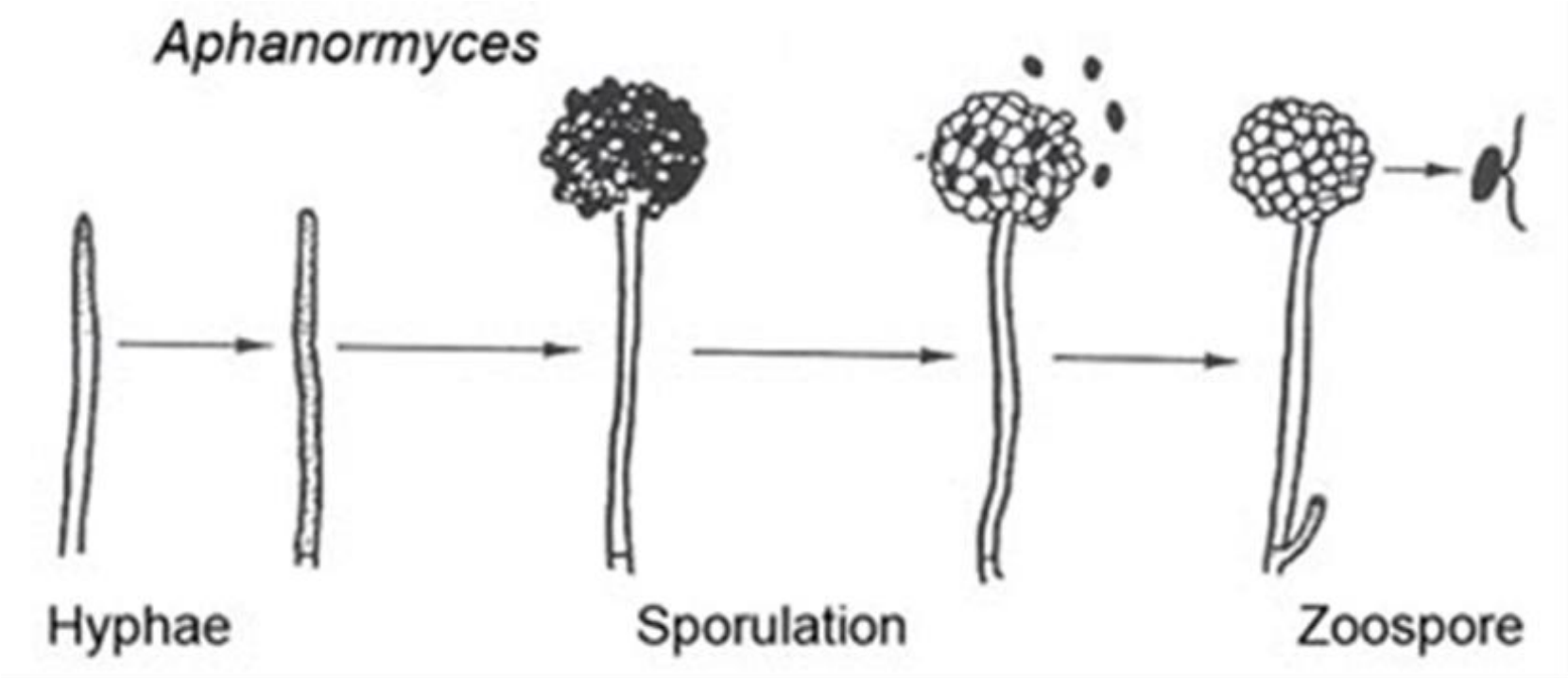
Muscle, severe ulcerations, inflammation and granulomas

Pathogenesis

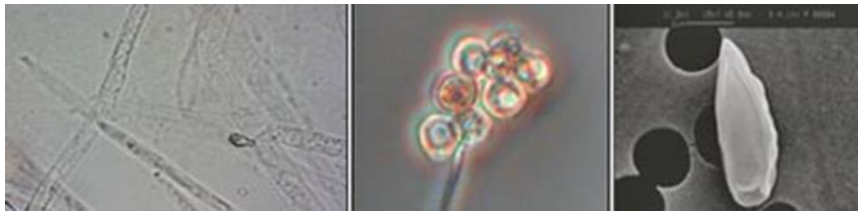
- *A. invadans* migrates towards the central nervous system, and then throughout the body, producing proteolytic enzymes which destroy muscle and other tissues
- Previously: arguments about etiology of EUS
  - In the United Kingdom, Lilley and Roberts provided convincing evidence that *A. invadans*, and not one or more other pathogens, is responsible for much of the characteristic pathology of EUS. They injected zoospores from 58 oomycete isolates intramuscularly into snakehead fish, *Channa striata*. These oomycetes comprised of: Aphanomyces strains isolated in Asian countries and Australia from EUS-affected fish; saprophytic *Aphanomyces*, *Achlya* and *Saprolegnia* spp. from infected waters; and oomycetes involved in other diseases of aquatic animals. Only the *Aphanomyces* strains isolated from fish affected by EUS, RSD or MG were able to grow invasively through the fish muscle and produce the distinctive EUS lesions. The snakehead-pathogenic strains were further distinguished from all the other oomycetes under comparison by their characteristic temperature-growth profile and inability to grow on certain selective media.

Pathogenesis

- *A. invadans* is aseptate and produces two zoospore forms, the secondary form being free-swimming and laterally biflagellate.
- No sexual reproductive structures have been observed in any of the isolates from EUS, MG or RSD outbreaks
  - The lack of sexual structures is considered to be a particularly common phenomenon among the more pathogenic members of the Saprolegniaceae
- *A. invadans* has a life cycle that consists of three different stages: hyphae, zoospore, and cyst. Changes in the aquatic environment (such as a change in salinity or water temperature) cause *A. invadans* to undergo asexual reproduction. During sporulation (asexual reproduction) thousands of swimming zoospores are released into the water. Infection occurs when these motile spores are attracted to the skin of fish. They penetrate the fish skin and germinate, forming oomycete hyphae
- If a host is not found, the zoospores become cysts and sink to the mud or sediment

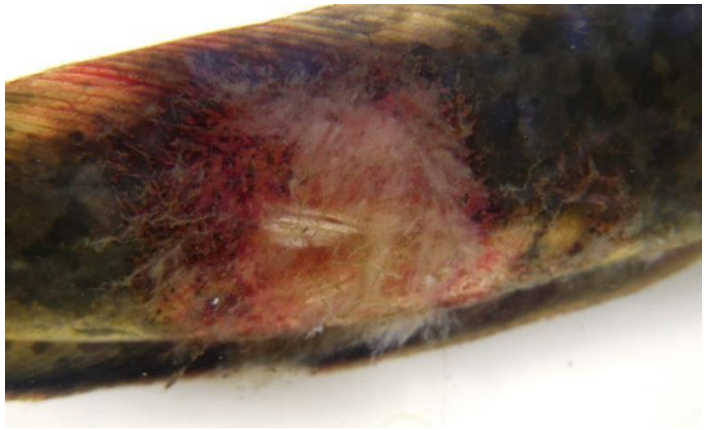


Life cycle of *A. invadans* (Lilley et al., 1998)



Life cycle of *A. invadans* showing hyphae, sporulation and zoospore stages

Hyphae invade widely into the surrounding skin and deeply into underlying muscle tissues, resulting in extensive, bloody ulceration and destruction of tissues. Initially, lesions may be characterised by the appearance of raised areas of induration and erythema. Subsequent skin erosion results in the formation of ulcerative lesions on the body. Varying sizes of ulcerative lesions may progress to necrotizing dermatitis resulting in deep dermal ulcers. If a host is not found, the zoospores become cysts and sink to the mud or sediment



Pathogenesis

- Pathogenicicty studies of *A. invadans* in *Oreochromis niloticus*, *O. andersonii* and *Barbus paludinosus* in Zambia revealed that *O. niloticus* does not succumb to infection with *A. invadans*, even following experimental infection in the lab. In the same experiment, *O. andersonii* and *Barbus paludinosus* showed severe clinical disease and, through re-isolation of *A. invadans* from infected tissue, Koch's postulates were confirmed

Risk Factors

- . Initiating factors which facilitate the entry of *A. invadans* are varied. In some locations infection is predisposed by cooler water temperatures, in others higher temperatures appear to trigger it. EUS has often occurred after periods of heavy rain.
- Low salinities appear crucial to the transmission of the pathogen.
- Potential causes of stressful environmental conditions include: temperature, eutrophication, sewage, metabolic products of fishes, industrial pollution and pesticides.
- Quality of water also appears to be significant from an aetiological point of view. Parameters like salinity, alkalinity, temperature, hardness and chloride concentration (many of which are seasonally variable) are known to predispose fish to attacks of EUS. Infected fish shows signs of improvement when transferred to clean freshwater ponds.
- *A. invadans* requires some predisposing condition of the host, such as debilitation or breach of the normal mucosal barriers along with favorable growth conditions before they can become established. Such conditions would include compromise of normal immune status of the fish (i.e., systemic and/or mucosal immunity). Some tank trials have been used to show that healthy, intact fish in aquaria exposed to *A. invadans* zoospores in water would not develop EUS lesions and that prior damage to skin was necessary before lesions could be induced in fish

Control

- Control in natural waters: probably impossible
- The most effective way of controlling the spread of the disease is to restrict the movement of fish (and/or contaminated material) from infected areas to uninfected areas
- In outbreaks occurring in small, closed water-bodies, liming water and improving water quality, together with removal of infected fish, is often effective in reducing mortalities and controlling the disease

Summary

EUS is caused by a fungal like organism, the oomycete *A. invadans*, and can have devastating social, economic and biodiversity impacts on affected communities. Poor environmental conditions predispose susceptible fish species to infection. *O. niloticus* has previously been shown to be resistant to infection with A. invadans. It must therefore be handled with care when co-cultured with susceptible fish species as it may probably harbor the pathogen and hence be a ‘carrier’ of the disease

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Thank you for your time