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Epizootic Ulcerative Syndrome (EUS) In Freshwater Fishes Infected By Fungal Genera *Aphanomyces* and *Alternaria,* Sindh, Pakistan

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Abstract: Epizootic ulcerative syndrome (EUS) is seriously damaging large numbers of commercially important fish species since last two decades. The disease is reported as activate especially from November to February at low ranges of light, temperature and salinity. Red spots, lesions and deep hemorrhagic abrasions were reported commonly on body of host fish. Mucus and surface memberanes were found contaminated by a number of fungal spores and hyphae of *Aphanomyces* and *alternaria.* Deep ulceration may cause the death of host fish.

Keywords: Freshwater Fishes, Fungal Genera *Aphanomyces* and *Alternaria,*

1. INTRODUCTION

Since last two decades, epizootic ulcerative syndrome (EUS) is seriously damaging large numbers of commercially important culturable fish species during the cold seasons in a large number of public waters of Sindh, Pakistan. This disease appears from end of autumn to early spring and affecting large numbers of fishes in ponds and stagnant water bodies. Epizootic ulcerative syndrome (EUS) is considered to be an infection due to fungal genus *Aphanomyces* and *Alternaria* characterized histological by penetrating hyphae surrounded by granulomatous inflammation. It is referred to be as Mycotic Granulomatoses (MG) and Red Spot Disease (RSD (Blazer *et al.,* 1999). On average, less than 10 zoospores of this fungus is enough to kill a fish, and even infection by just a single zoospore can result in ulcerous lesions that can lead to mortality (Mohan and Shankar, 1995; Lilley *et al.,* 1998; Thompson *et al.,* 1999; Johnson *et al.,* 2004). Fishes infected with *Aphanomyces* that develops ulcerous lesions which ultimately lead to extensive tissue necrosis (Blazer *et al.,* 1999). The Thatta is one of rich districts in Sindh and famous by aquaculture point of view, besides the Keenjhar and Haleji freshwater lakes, the river Indus also flow across the district. About 3200 fish ponds, 1000 public waters i-e natural lakes, canals and semi natural depressions are under practice for fish cultivation. Thousands of metric tons of fish production in Thatta district per annum is also being harvested. Majority of fish farmers are practicing the poly culture system of carps species belonging to family Cyprinidae.

The present study expressed the prevalence of fungal species that caused the ulcerate lesions in fishes and become the cause of death. The objectives of this study were to examine infectivity of fish and preventive measures to save the fish from this chronic disease.

2. MATERIALS AND METHODS

The effected fish farms were surveyed during November 2011 to February 2012. Water and effected fish samples were collected from ponds of public waters of district Thatta, preserved in sterilized plastic bottles and poly-thin bags brought to the laboratories at Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro and Fish Hatchery Chilya Thatta. Physico-chemical parameters of water especially temperature, pH and salinity (‰) were examined on spot using the mercury thermo meter; pH meter model-98107 and salinity meter model M-98302. The microbiological samples from ponds were collected with the help of nano-plankton net (mesh size 55µm) especially for analysis of fungal spores and preserved in 5% formalin solution. Infected fishes (alive) were collected with the help of hand nets transferred to antiseptic nursery size 25x50 ft. The sample of mucus and tissues from lesion's part of affected fish were collected with fine sterilized blade studied with the help of DCM 350 (0.1 USB) under the trinocular microscope at x400 and x1000. Therapeutic action was immediately started externally using mixture of antibacterial chemical solutions (sprit 50%, Benzebiotic 30% and Piodine 20%) and fatty ointments. Each fish was treated once time in a day. Oxytetracycline and Diclofenic sodium injections 0.4 cc dosage per kg of body weight of fish were given/ day for a week.

3. RESULTS

The effected live fishes were collected and placed under the first-aid. The mucus and surface membranes from lesion's parts of effected fish were

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examined under the microscope and observed activeness of fungal motile spores of *Aphanomyces* sp. **(Fig.1).** 50 liter volume of water samples were collected from effected habitats strained through plankton net (no. 25, mesh 55µ) that proved the presence of 5 to 7 number of spores and hyphae of *Aphanomyces* and *Alternaria*. The *Aphanomyces* motile spores were dark brown in color, oval shaped 2-3 µm in diameter, while the shape of *Alternaria* spores were rounded frontal and last part was pea shaped and in hyphae the cells were arranged in a graded series of diminishing size. Length measurement of hyphae was 67-72 µm **(Fig. 2-3**). Five to ten numbers of small and large sized fish were found in dead form in ponds affected by epizootic ulcerative syndrome (EUS) during cold weather at temperature ranges 11-17 ºC and salinity 0.05 to 0.2ppt on daily basis. Mislaid of scales and skin were also found at early stage, afterward small lesions and red spots overall on the body were noted **(Fig. 4).** After 10 days gape the effected waters were again surveyed and investigated the position of disease, thus many ulcer spots were found on different parts of the body even heads and in few specimens ulcers were seen to extend down to abdominal cavity sides **(Fig.5-8).** Each fish was treated once time in a day. Oxytetracycline and Diclofenic sodium injections 0.4 cc dosage per kg of body weight of fish were given/ day for a week. 60% positive response was received by the application of fatty ointments.

4. DISCUSSION

The syndrome has been reported broad-based occurring in culturable species in Southeast Asia (Roberts *et al.,* 1993), Japan (Egusa and Masuda, 1971; Hatai *et al.,* 1977; Wada *et al.,* 1996) and Australia (Fraser *et al.,* 1992; Lilley and Roberts, 1997). This most destructive disease occurring both fresh and brackish water farmed and wild fish which caused major fish losses in many countries for over last three decades (Baldock *et al.,* 2005).

The similar epizootic ulcerative syndrome was reported on the body of carps *Catla catla, Cirrhinus mrigala, Hypomichthyces molitrix, Hypophthalmichthys nobilis* and snake head *Channa marulius* in public waters of Sindh, Pakistan, during cold seasons and low salinity ranges. EUS disease in fishes was reported from waters having low light, temperature and salinity. The spores of *Aphanomyces* and *Alternaria* developed abundantly in waters due to use of unhygienic organic dung and old feed and fishes are affected severely. The *Alternaria* species are commonly found in decay and decomposed materials. The spores are air-born but commonly found in the soil and water. The human health disorders can be caused by *Alternaria*, which grow on skin and mucous membranes, including on the eyeballs and within the respiratory tract (Wikipedia encyclopedia). The same situations were reported in the carp fishes by damaging the skin, gills and eyeball. Infections happened by motile spores of fungus in the water captivated and enter to the skin of fish and then germinated, forming fungal filaments or hyphae. A variety of saprophytic *Aphanomyces*, *Achlya* and *Saprolegnia* species isolated from the surface of EUS-affected fish or from infected waters, and Oomycete fungi involved in other diseases of aquatic animals (Lilley, 1997). Disease was reported active form cold seasons in low temperature during winter to early spring and affecting large numbers of fishes in ponds and stagnant public waters even reported in estuarine fishes like mullets (Mckenzie and Hall, 1976). Epizootic condition in freshwater fishes a complex infectious aetiology character caused by the presence of invasive *Aphanomyces* infection and necrotising ulcerative lesions typically leading to a granulomatous response (Roberts *et al.,* 1993). The same situations were observed in effected fishes found in Sindh.

Tilapia is considered as one of the resistant species to EUS (Lilley *et al.,* 1998). Pradhan et al (2008) conducted experiments with *Aphanomyces invadans* in advanced fingerlings of four different carp species for 12 days and found 100% mortality rate in major carps. In the present investigation the mass mortality and infections were recorded in carp and snake head species. Fatty antibiotic ointments gave positive results from treatment and cure point of view.

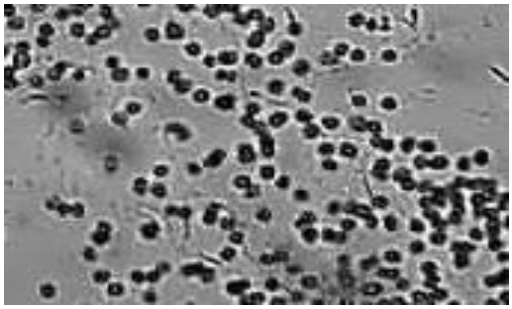


Fig. 1. *Aphanomyces* zoospores collected from mucus lesion area of carp fish

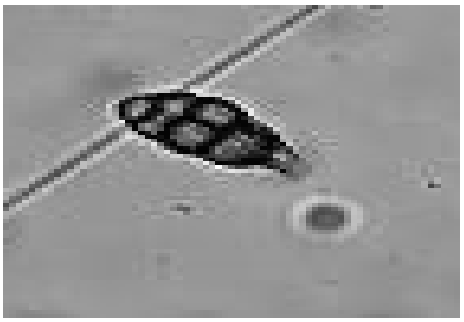


Fig. 2. *Alternaria* spore



Fig. 3. *Alternaria* hyphae

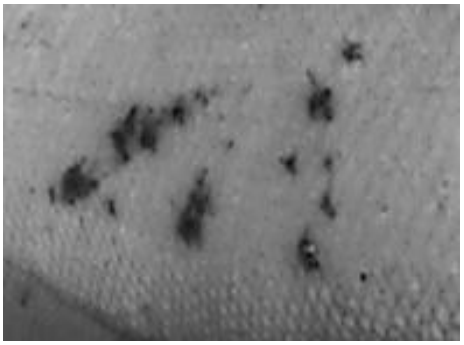


Fig.4. Red-spots on host fish

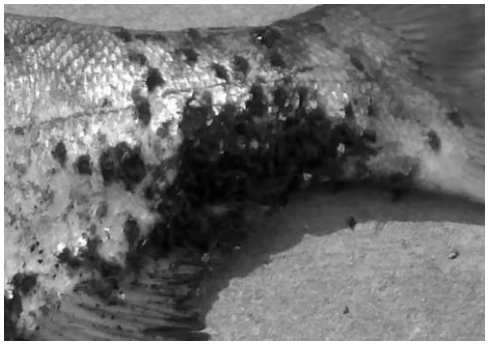


Fig.5. Deep hemorrhage ulceration

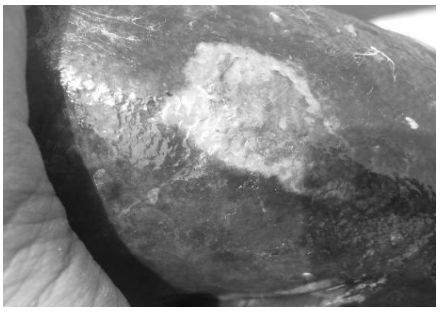


Fig.6. Lesion on head region of fish.

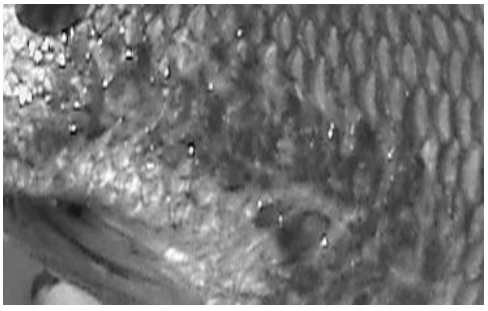


Fig.7. Ulcerative lesion near tail of fish.

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