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Treatment of Epizootic Ulcerative Syndrome (EUS) in *Anabas testudineus* with an Alkali-Soluble Fraction from Paddy Husk

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ABSTRACT

Epizootic ulcerative syndrome (EUS) is a fish disease characterized by the presence of severe, open dermal ulcers on the head, mid-body, and dorsal regions of the fish. The disease is widely prevalent throughout Bangladesh, the major species of fish reportedly affected being carps, *Anabas testudineus*, *Heteropneustes fossilis* and fish belonging to the *Channa* and *Puntius* species. We investigated the effect of feeding an alkali-soluble fraction from paddy husk (ASFPH) to *Anabas testudineus* manifesting symptoms of EUS, and report that symptoms of EUS start reducing within 72 hours and totally disappear within 60 days of oral administration of a diet containing 0.1% ASFPH to EUS-affected *Anabas testudineus*.

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INTRODUCTION

Since the 1980s, an epizootic fish disease has been affecting various fish species throughout the world, more so in South and Southeast Asian countries like Bangladesh, India, Pakistan and the Philippines. The disease known as epizootic ulcerative syndrome (EUS) is characterized by the presence of severe, open dermal ulcers on the head, mid-body, and dorsal regions of the fish (McGarey *et al*, 1991), and is indistinguishable from red spot disease first observed in Eastern Australia in 1972, and from mycotic granulomatosis, which was first reported in Japan in 1971. Since then EUS has spread its range through Papua New Guinea and EUS-like syndrome in fish species has been reported from as far away as USA.

EUS has been reported for *Puntius ticto* in Southern Assam of India (Dey *et al*., 2009). The Zambezi floodplain and Zambezi River basin has also reported presence of EUS-infected fish (Choongo *et al*., 2009; Songe *et al*., 2012). A total of 16 fish species was found to be affected in the Zambezi River basin, including *Clarias ngamensis*, *Clarias gariepinus*, *Barbus poechii*, *Tilapia sparrmanii*, *Serranochromis angusticeps*, *Brycinus lateralis*, *Micralestes acutidens*, *Sargochromis carlottae*, *Hydrocynus vittatus*, *Phryngochromis acuticeps*, *Schilbe intermedius*, *Hepsetus odoe*, *Labeo lunatus*, *Oreochromis andersonii*, *Barbus unitaeniatus*, and *Barbus paludinosus* (Songe *et al*., 2012). Epizootic ulcerative syndrome caused by *Aphanomyces invadans* has been reported in captive bullseye snakehead *Channa marulius* collected from south Florida, USA (Saylor *et al*., 2010). EUS and *Aphanomyces invadans* have been reported in wild-caught bony bream (*Nematalosa erebi*) from the Darling River near Bourke, in New South Wales, Australia (Go *et al*., 2012).

The causative agent for EUS is most possibly the peronosporomycete fungal species *Aphanomyces invadans* (Lilley *et al*, 2003). The fungus has been isolated from Atlantic menhaden (*Brevoortia tyrannus*) along the eastern seaboard of USA suffering from ulcerative mycosis, under which the fish develop lesions similar to EUS. Other fish species, when inoculated with the fungus develop lesions or reddened areas include the hogchoker (*Trinectus maculatus*), striped killifish (*Fundulus majalis*), and mummichog (*Fundulus heteroclitus*) (Johnson *et al*, 2004). Vaccines prepared from *Aphanomyces invadans* has been shown to reduce mortality in EUS-infected *Catla catla* (Saikia and Kamilya, 2012). However, the fungus is unable to attack healthy fish.

It appears that some sort of epidermal damage arising out of environmental stress or other factors enables the fungus to invade and cause EUS. As an example, EUS has been observed to develop in empire gudgeon (*Hypseleotris compressa*), an Australian eleotrid following a combination of fungal infection and sublethal

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exposure to runoff from acid sulfate soils (Callinan *et al*, 2005). EUS may be followed by a host of other opportunistic infections by bacteria and viruses. For instance, chemoautotrophic nocardioform actinomycetic organisms have been repeatedly isolated from different varieties of fish affected with EUS lesions of dermis, muscle, subcutaneous tissues and internal organs (Chakrabarty *et al*, 1991a; Chakrabarty and Dastidar, 1991b). These organisms have been shown to produce pathogenic effects in mice similar to that of the rat leprosy bacillus (Mukherjee *et al*, 1995). They are reportedly also similar to human tissue derived *Mycobacterium leprae* cells (Chakrabarty *et al*, 1996).

Other organisms (possibly opportunistic and causing adventitious infections) isolated from diseased areas of EUS-affected fish include *Aeromonas hydrophila*, *Aeromonas sobria*, *Vibrio anguillarum*, *Vibrio vulnificus*, *Alteromonas putrefaciens*, *Pseudomonas fluorescens*, and *Plesiomonas shigelloides* (McGarey *et al*, 1991; Rahman *et al*, 2002; Mastan and Qureshi, 2001). *Aeromonads* and *Pseudomonads* have been implicated in EUS in *Anabas testudineus* (Saha and Pal, 2002). A fish virus has also been isolated from severely lesioned EUS-affected snakehead fish, *Ophicephalus striatus* (also known as *Channa striata*) in the Philippines (Lio-Po *et al*, 2000; Lio-Po *et al*, 2003). The virus could possibly be the snakehead reovirus (John *et al*, 2001). *Pfiesteria piscicida* and other *Pfiesteria*-like dinoflagellates have also been implicated as the causative agent for the lesions, particularly mycotic granulomatous ulcers in Atlantic menhaden (Kane *et al*, 2000). Rhabdoviruses and birnaviruses have also been implicated in EUS. One reovirus and a ranavirus have also been isolated from ulcerated fishes of which the ranavirus was capable of reproducing the clinical signs of EUS (John and George, 2012).

The antibiotics oxytetracycline and chloramphenicol have been recommended to treat EUS (Saha and Pal, 2002). However, treatment of EUS-affected fish with antibiotics cannot be an effective solution for a number of reasons- it is impossible to treat diseased fish in open water bodies; even in medium to large ponds, administration of antibiotics through diet may become prohibitively expensive; moreover, consumption of antibiotic-treated fish may lead to development of antibiotic-resistant pathogenic organisms in human beings. Hence a cheaper method must be found, especially so for fish farmers in Bangladesh, who traditionally are small rural cultivators.

Administration of immunostimulating agents to fish can perhaps be a better way to combat EUS. A macromolecular compound beta-1,3-1,6-glucan, isolated from yeast cell walls but also present in bran of various cereals has been shown to induce non-specific disease resistance in the saltwater tiger shrimp *Penaeus monodon*, when administered through diet or when shrimps were immersed in glucan suspension prior to culturing. The compound acts as an immunostimulant and treatment of shrimps with glucan led to resistance against infection by *Vibrio vulnificus* and white spot syndrome virus (Song *et al*, 1997). Beta-1,3-Glucan, isolated from *Schizophyllum commune* reportedly enhanced survival of brooder *Penaeus monodon* (Chang *et al*, 2000). When orally administered at a dose of 10g glucan/kg diet, glucan derived from *Schizophyllum commune* enhanced the immune system and improved the survival rate of white spot syndrome virus-affected tiger shrimp (Chang *et al*, 2003). In *in vitro* experiments, glucan isolated from spent brewer's yeast significantly enhanced phenoloxidase activity of tiger shrimp hemolymph and thus demonstrated potential immunostimulating properties (Suphantharika *et al*, 2003). Paddy husk is widely available in Bangladesh and reportedly contains lignins, which may act as immunostimulants (Sakagami *et al*., 2011). We therefore examined the effect of oral administration of an alkali-soluble fraction (ASFPH) extracted from paddy husk on EUS-affected *Anabas testudineus*.

MATERIALS AND METHODS

Extraction of ASFPH:

ASFPH was extracted from paddy husk (containing about 5% bran) procured locally. Briefly, husk was boiled in 5 volumes of 1N NaOH at 90^{o}C for 1h. The NaOH extract was cooled to room temperature, filtered, and followed by acidification of the filtrate with HCl. The filtrate was stirred and left at room temperature, following which the precipitate that formed was collected, washed thrice with deionized distilled water and once with absolute ethanol. The precipitate was next dried in an oven at 60^{o}C. The resultant product (amounting to about 5-10% w/w of the starting material) was found to be reddish-brown in color and used for further studies.

Anabas testudineus:

Anabas testudineus (local name: koi) was procured from a local farmer who reported diseased koi in his pond. During procurement it was ensured that all fish were suffering from EUS. Through appropriate visual selection, fish were chosen which showed equivalent amounts of EUS manifestations as exhibited by lesions on the skin, fungal like growth on the head and rotting of tail sections. The fish was divided into two groups (I and II). Group I (control) was fed a diet without ASFPH, and Group II was fed a diet containing in addition, 0.1% ASFPH. Fish were maintained in 4 X 2 X 3 feet glass tanks containing 180 liter water and 10 fish per tank (three tanks per group). The water was changed every 2 days. Fish were fed *ad libitum*, and diets were administered from Day 1 following procurement.

Advances in Natural and Applied Sciences, 8(9) August 2014, Pages: 7-12

Composition of diet:

The composition of the basic diet (per 500 g) was fish meal (100g), rice polishing (100g), wheat (200g), soybean meal (100g), vitamin C (0.5g), molasses (50g), ASFPH/carboxymethyl cellulose (5g). The individual components were mixed by blending, followed by passage through a pelleting machine to form pellets. The pellets were dried prior to feeding fish.

RESULTS AND DISCUSSION

Fish administered a diet without ASFPH showed progressive worsening of disease conditions, as manifested by more and more fungal like growth on the head and rotting of body parts, especially the fin and tail sections. There was a substantial increase in the mortality rates of Group I fish. 50% of Group I fish died within the first ten days, and a total of 28 fish died within another twenty days. Even after 60 days into the experiment, the remaining two fish did not show any marked improvement in the lesions; however, they survived and their conditions did not worsen. The overall mortality rate was 96.7%.

Amongst the Group II fish, which were fed a diet containing 0.1% ASFPH, 4 fish died within the first ten days followed by the death of 3 more fish within another twenty days. The rest of the fish survived. The overall mortality rate was 11.7%. The surviving fish demonstrated a gradual reduction of diseased conditions, which in general showed the following pattern. First, the fungal protrusions on the head and fin regions (Figures 1 and 2) fell off within 72 hours of being fed an ASFPH-containing diet, leaving reddish epidermal open scar tissue behind (Figure 3). The scars gradually healed and the red color lessened within 20 days following administration of the ASFPH diet (Figure 4). Figure 5 shows that by the end of 60 days, all scars have completely healed; the epidermis only showed faint red spots at the original lesions and skin color has returned to normal.

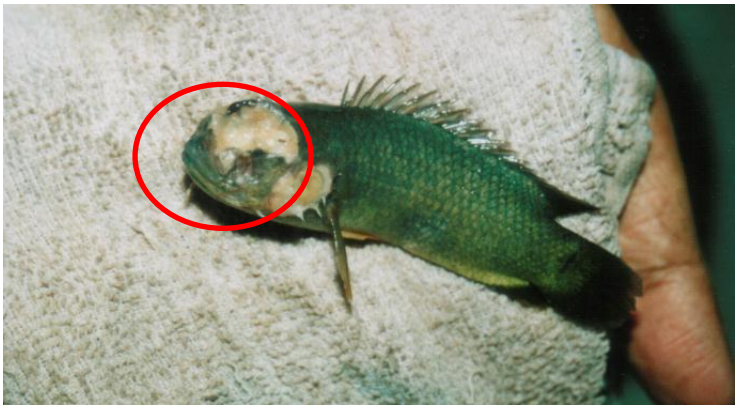

Fig. 1: *Anabas testudineus* showing typical symptoms of EUS.

The picture taken on Day 1 following procurement of fish shows fungal-like protrusions on the head region around the eyes (circled in red). All fish with EUS also demonstrated sluggish swimming behavior and clustered around one spot of the fish tank. The picture is typical of the rest of the fish with EUS.

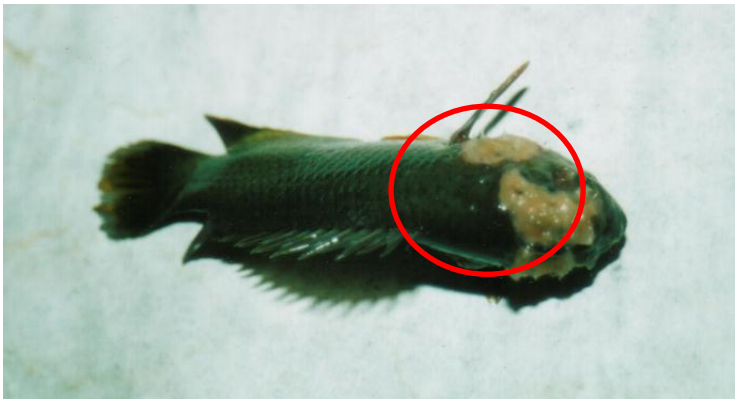

Fig. 2: *Anabas testudineus* showing typical symptoms of EUS.

View of the same fish with EUS taken from a different angle. The picture was taken on Day 1 following procurement of fish and shows fungal-like protrusions on the head region around the eyes (circled in red). Note also that the pectoral fin also has a reddish appearance.

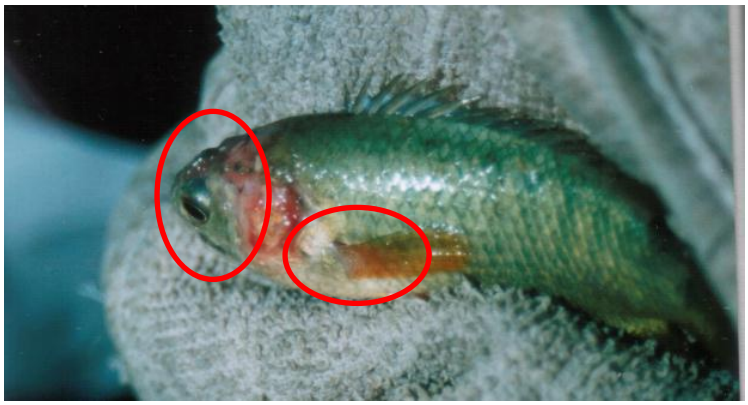

Fig. 3: *Anabas testudineus* following 72 hours of administration of 0.1% ASFPH-containing diet.

The picture shows a typical EUS-affected fish 72 hours following administration of a 0.1% ASFPH-containing diet. Note that the fungus-like protrusions has fallen off following ASFPH feeding, leaving open epidermal scars, which are reddish in appearance (circled in red). Note also the red pectoral fin (circled in red).

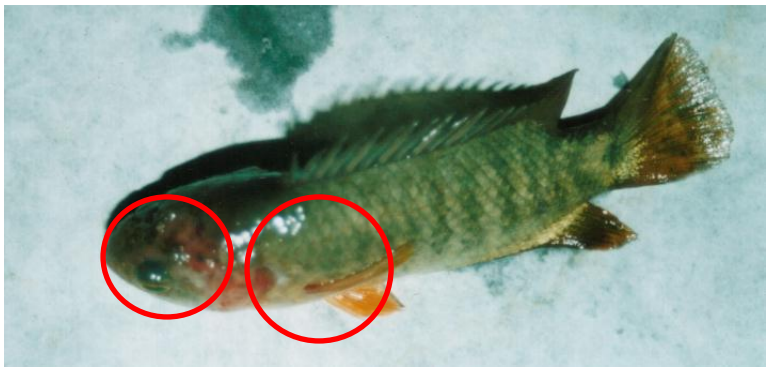

Fig. 4: *Anabas testudineus* following 20 days of administration of 0.1% ASFPH-containing diet.

The picture shows a typical EUS-affected fish 20 days following administration of a 0.1% ASFPH-containing diet. Note that the fungus-like protrusions has fallen off following ASFPH feeding, the raw epidermal scars have partially healed with re-growth of normal epidermis (circled in red). Note also the red pectoral fin color has also diminished and turned to a more orange hue (circled in red).

Fig. 5: *Anabas testudineus* following 60 days of administration of 0.1% ASFPH-containing diet.
Note that all lesions have completely disappeared and normal epidermal color has returned. There is only a faint reddish hue present (circled in red) and all signs of fungus are gone.

It is evident from the above results that ASFPH from paddy husk had a positive effect on the curing of EUS-affected *Anabas testudineus*. This effect can be attributed to stimulation of fish immune system and concomitant increase in cure of the disease. Alternately, ASFPH can have possible antimicrobial activity and so gave a therapeutic effect. This may involve an inhibitory effect on the growth of the fungus, assuming that the infective agent is *Aphanomyces invadans*. Irrespective of the actual mechanisms involved, it appears from the present study that ASFPH isolated from paddy husk and administered through fish diet can be a cheap and effective cure for EUS, which is devastating a number of fish species of Bangladesh and the rest of the world.

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