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Histopathological studies of EUS affected shing, *Heteropneustes fossilis* (Bloch) from a fish farm of Mymensingh, Bangladesh

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Abstract
An investigation was carried out to observe histopathological changes in liver and kidney of suspected epizootic ulcerative syndrome (EUS)-affected shing fish, *Heteropneustes fossilis* (Bloch) collected from the "Agro-3 fish farm" situated at Boilor, Trishal, Mymensingh. Focal necrosis, haemorrhages and atrophy of the sinusoidal region were observed in the liver tissue. Fungal granulomas were found both in liver and kidney. In some cases fatty depositions were observed in all over the hepatic tissue. Degeneration and necrosis of renal tubular epithelial cells were also occurred. Missing of glomerulus and necrosis surrounding the Bowman's capsule in the kidney tissue were found.

Key words: *Heteropneustes fossilis*, EUS, Histopathology

Introduction

Among the commercially important fishes of Bangladesh *Heteropneustes fossilis*, locally known as "shing", is an important air breathing catfish in Bangladesh and generally grows in pond, lake, baor, beels and floodplains with natural care. Shing fish has been reported to be affected by some metazoan parasitic diseases (Sanaullah 1976) and bacterial diseases (Sahoo and Mukherjee 1997). In India, *Gyrodactylus neonephrotus* was detected from the skin of *H. fossilis* by Singh and Agarwal (1994). Srivastava *et al.* (1998) stated the toxicity of malachite green in liver of a shing during the pre-spawning phase. Detrimental effects on the liver cells, including hypertrophy and vacuolization, followed by necrosis and cirrohosis were observed at acute, sub-acute and sublethal concentrations in both the short and long term treatments. Sahoo and Mukherjee (1997) detected three Gram negative bacterial pathogens as *Aeromoas hydrophila, Edwardsiella tarda* and *Haemophilus piscicum* from shing fish *Heteropneustes fossilis*. The epizootic ulcerative syndrome (EUS) have been detected in many fishes of Asia Pacific region (Lilley *et al.* 1992). In Bangladesh the EUS have also been found in many natural and culture fishes by different authors (Barua 1989, Ahmed and Rab 1995, Chowdhury *et al.* 1996). Sahoo *et al.* (1998) detected the ulcer disease in the shing fish for the first time in India. Barua (1989) mentioned shing fish in his list of susceptible fishes to EUS. But

until 2006, this fish was not found to develop ulcer type disease in Bangladesh. However, in September, 2006, the fish was found to be affected by EUS like ulcer disease in a fish farm named "Agro-3 fish farm" in Bailor, Mymensingh.

In all countries detection of fungal granuloma together with other histopathological findings have been proved to be the confirmatory tests of the etiological agent *Aphanomyces invedans* in EUS affected fishes, includng Bangladesh by different autrhors. Haque *et al.* (1999) observed the fungal granuloma together with necrosis, haemorrhage and pyknotic cells in EUS affected catla fish. So the above diseased fish were undergone histopathological investigations in order to confirm the etiology of the ulcer type lesions, in one side and in another side, bacteriological investigations were done in order to understand the involvement of any bacteria with those lesions. The present paper deals with the histopathological findings of the fish. Report of their bacteriological investigation will be published elsewhere.

Materials and methods

Study area and duration

A fish farm namely "Agro-3 Fish Farm" was selected for the present experiment that was situated at Boilor, Trishal, Mymensingh. Duration of the study was September, 2006 to January, 2007.

Collection of sample

A total of 20 *Heteropneustes fossilis* (Shing) were selected as the experimental fish. They were collected from one of the ponds of the above farm, in which the fish were suffering from suspected EUS like lesions and were having severe mortality. Such diseased fish were caught by using seine net during sampling. No dead fish were taken. After netting, the fish were taken in a plastic bucket with pond water and immediately brought to the fish disease laboratory, Bangladesh Agricultural University, for study.

Fixation and preservation

Samples of liver and kidney were collected from each fish by a sharp scalpel and forceps for histopathological study. An 1 cm^3 portions of each organ, from each fish were taken and fixed in 10% neutral buffered formalin. The amount of fixative was 10 times to bulk of tissue fixed (Humason 1979). After at least 10 days of fixation, the samples were trimmed for histopathological preparations.

Histopathological procedure

The fixed samples were taken out with forceps from the vial and placed separately in a perforated plastic holder which was covered by perforated still plates. Marking was done with dark pencil (2B) in perforated plastic holder. The samples (blocks) were then arranged in a still rack and placed in an automatic tissue processor for dehydration, clearing and infiltration (SHANDON, CITADEL 1000). Alcoholic series of higher concentrations, xylene and paraffin wax were used in the processor maintaining at

various time schedules. After tissue processing (21 hours), the samples were embedded with melted wax on perforated plastic holder and still mold. The solid blocks were placed in a refrigerator (deep freeze) for half an hour and paraffin blocks were separated from the still molds. Surface and side of paraffin blocks were trimmed with the help of scalpel and microtome (Leica, JUNG RM 2035). Blocks having hard tissue before sectioning were decalcified by dissolving the surface of blocks in water for one hour followed by washing rapidly. The blocks were placed again in deep freeze for 30 minutes. Sections were taken from the blocks at a thickness of 5 micrometers. The ribbon with section was placed on a water bath at a temperature of 40°C, which were finally picked up over glass slides. The sections were then stained with haematoxyline and eosin and were mounted with Canada balsam. The stained slides were then examined under a compound microscope (Olympus). Photomicrographs were done by using a photomicroscope (OLYMPUS, Model CHS, Japan).

Results and discussion

From the severe outbreak of ulcer type disease in shing fish of the Agro-3 farm, first time in Bangladesh, round ulcers were observed on body surface. No such ulcer type lesions in *Heteropneustes fossilis* were found in Bangladesh prior to this occassion. However, Sahoo *et al.* (1998) found an outbreak of ulcer disease of shing fish in India.

The results of the histopathological study revealed necrosis and atrophy of the sinusoidal region in the liver of the infected fish (Fig. 1). Focal necrosis and haemorrhages were occurred in the liver tissue (Fig. 2). Fungal granulomas were found in liver tissue of the shing which proved that the causative etiology of the ulcerative disease was *Aphanomyces* like fungal pathogen. Prominent vaculations were also observed (Fig. 3). Fatty deposition was found in the whole hepatic tissue (Fig. 4). Degeneration of renal tubules were detected in the haematopoietic tissue (Fig. 5). Necrosis and vacoulation of renal tubular epithelial cells with fungal granuloma were present in kidney tissue (Fig. 6). Missing of glomerulus and necrosis surrounding the Bowman's capsule were found (Fig. 7). Hemorrhages occurred in the whole kidney tissue with fungal ganuloma (Fig. 8) ensuring the disease as EUS. In another part of this investigation (Hasan 2007), *A. hydrophila* bacteria were isolated from the above ulcers. So, to compare the present natural histopathology with that of experimental one by *A. hydrophila*, an experimental histopathplogical investigation of the shing fish was done by Islam (2006). He found that *A. hydrophila* caused focal necrosis in haematopoietic tissue together with hemorrhage and atrophy. Infected liver showed pathology like massive atrophy, haemorrhages, necrotic hepatocytes, focal necrosis and atrophy of hepatic sinusoids represented by necrosis of the sinusoidal lining cells. *A. hydrophila* cells were distributed all over the hepatic tissue.

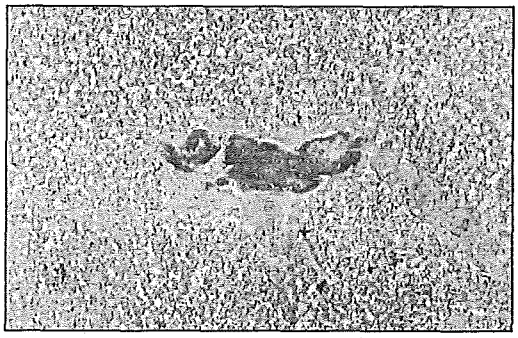

Fig. 1. Necrosis (n) and atrophy (↗) of the sinusoidal region in liver of naturally infected shing. H & E (×125).

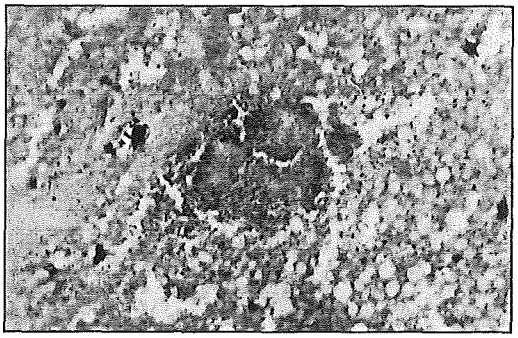

Fig. 2. Focal necrosis (↗) characterized by haemorrhagic lesion in the liver of a naturally infected shing fish. H & E (×440)

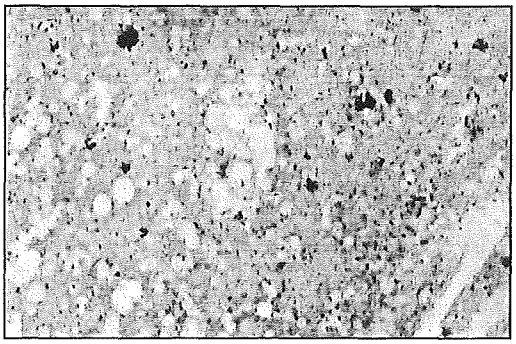

Fig. 3. Fungal granuloma (↗) were found in the liver with vacuolation (v) in a shing fish. H & E (×440).

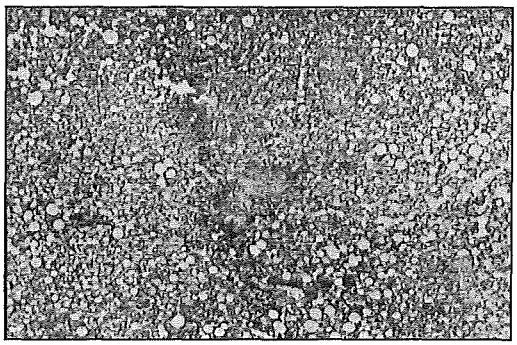

Fig. 4. Focal necrosis (↗), hemorrrhages (h) and fatty deposition occured in the liver tissue of a shing fish. H & E (×125).

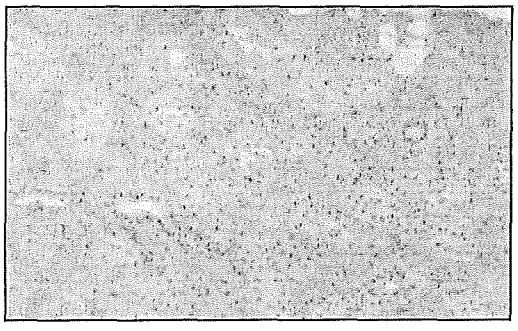

Fig. 5. Degeneration of renal tubules (r) were detected in the haemato-poietic tissue. H & E (×440).

Fig 6. Necrosis (n) and vacoulation (v) of renal tubular epithelial cells with fungal granuloma (↗) in kidney tissue. H & E (×440).

Fig. 7. Missing of glomerulas (↗) and also necrosis (n) surrounding the Bowman's capsule in the haematopoietic tissue. H & E (×125).

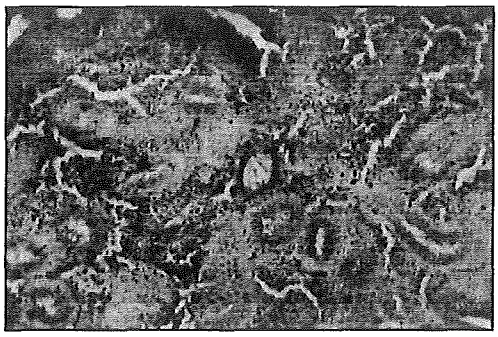

Fig. 8. Haemorrhages (h) occurred in whole kidney tissue with fungal granuloma (↗) of infected shing fish. H & E (×440).

The findings of Islam (2006) were considered to be similar with the present natural histopathological changes of the shing fish. So it may be concluded that the present histopathological changes might be the concurrent effect of both the fungal pathogen (*Aphanomyces*) and the bacteria *A. hydrophila.* EUS diseases of many fishes were found to be caused by both the above pathogens by many authors in different fishes. Faruk *et al.* (2002) studied some diseased carps and varying degree of pathology was noticed in the kidney of fishes, which included degeneration and necrosis of kidney tubules, vacuole, tubular granuloma, haemotopoietic necrosis and encystment of parasite in the kidney. Ram and Singh (1988) reported varying degrees of pathological changes from *Channa punctatus* including cytoplasmolysis, nuclear pyknosis, and necrosis leading to complete degeneration of hepatocytes. Ventura and Grizzle (1988) observed lesions associated with natural and experimental infections of *A. hydrophila* in channel catfish, *Ictalurus punctatus.*

From the present study it was understood that the natural EUS lesions in concern of shing was caused by the fungus *A. invadans* together with *A. hydrophila* bacteria because the bacteria *A. hydrophila* was isolated from these lesions (Hasan 2007) and fungal granuloma were detected through histopathology. Akter *et al.* (2006) found well developed fungal granuloma from the kidney of EUS affected *Macrognathus aculeatus.* The fungal granuloma in the liver of *Cirrhinus mrigala* were confirmed by Hatai *et al.* (1994) and Roberts *et al.* (1994) to be proof of EUS by *A. invadans.* Hoque *et al.* (1999) observed necrosis, haemorhage, pyknotic cells and numerous fungal granuloma with some hyphae in kidney tissue of *Catla catla.* Islam (2006) did not find fungal granuloma in the shing fish because he infected shing fish artificially only by *A. hydrophila.* So pathology observed in naturally infected shing fish were understood to be caused by *A. hydrophila* together with *A. invadans* as supported by the findings of Islam (2006) in his artificial infection experiment. From the present study, it may be concluded that culturists may adopt proper management practices to avoid EUS and to get rid of the infection by such serious bacterial pathogen like *A. hydrophila.*

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