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HISTOPATHOLOGICAL STUDIES OF EXPERIMENTALLY INFECTED SHING, *Heteropneustes fossilis* WITH *Aeromonas hydrophila* BACTERIA

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ABSTRACT

Heteropneustes fossilis (shing) of 35g body weight were experimentally infected with *Aeromonas hydrophila* by two different methods: viz. intraperitonial and intramuscular injection. A standard dose of infection (6.4 × 10^7 CFU/ml) was selected based on predetermined LD_{50}. Each method gave rise to the mortality of shing up to 85%. Clinical signs of fish included injury, hemorrhage and large ulcerative lesions on the body at the injected area. In kidney, liver and intestine pathologically, massive atrophy and focal necrosis were found. Hemorrhage, Vacuolation and atrophy of hepatic sinusoids represented by necrosis of the sinusoidal lining cells, degeneration of hepatic tissue and distribution of bacterial cell all over the tissue were found in liver. Atrophy, hemorrhage, villi missing and missing of epithelium were found in intestine of the experimentally infected shing. Bacterial cells were distributed in the whole hematopoietic tissue including the renal tubules. Tissue abscess characterized by focal necrosis, hemorrhage and Vacuolation were also found in the kidney of the infected shing. But the above symptoms were not found in the organs of the apparently healthy shing species.

Key words : *Aeromonas*, Experimental infection, Histopathology, Shing

INTRODUCTION

Heteropneustes fossilis (Bloch) locally known as "shing" is an important air breathing catfish in Bangladesh. The nutritive and medicinal value of this fish has been recognized for time immemorial. It has been drawing the attention of more and more fish farmers in Bangladesh day by day due to its high market value, profitable culture and hardy nature (Saha *et al*. 1998). The fish was supposed to be strongly resistant to many infectious diseases like Epizootic Ulcerative Syndrome (EUS). However, recently, the fish was reported to be affected by EUS like ulcer type lesions in an established fish farm of Mymensingh from which suspective *Aeromonas* like bacteria were isolated from the lesion (Hasan, 2007). Roberts *et al*. (1989) also reported that *Aeromonas hydrophila* and *A. sobria* were dominant bacteria in the EUS affected fishes. Rahman and Chowdhury (1996) detected 72-82% *Aeromonas* sp. in the kidney of ulcer diseased farmed fishes. Diseased catfish, with external signs or having latent infection could be diagnosed through histopathological study of various organs like skin, muscle, gill, kidney and liver as practiced by many workers (Ventura and Grizzle, 1988; Grizzle and Kiryu, 1993; Ruksana, 1998). Histopathological technique is one of the most important procedures for

disease diagnosis in fishes. So, the present study was undertaken to relate the abundance of *A. hydrophila* bacteria with the health hazard of shing fish and to study the histopathological changes in kidney, liver and intestine of shing fish through experimental infection.

MATERIALS AND METHODS

Study area and duration

The experiment was conducted for a period of six months, from July 2006 to December 2006, in the fish disease laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh.

Experimental fish

The catfish of average body weight of 35 g were collected from the stock pond of the Faculty. Prior to experiment, all the fish were acclimatized in 12 rectangular glass aquaria for seven days at wet laboratory.

Preparation of bacterial suspension

Aeromonas hydrophila were purely cultured on TSA agar at 25°C for 48 hours prior to the experiment. An amount of 52 mg of fresh culture of the bacteria was carefully scraped and mixed with 4 ml sterile physiological saline (0.85% NaCl = PS) and desired dilutions were prepared by serial decimal dilution method.

Experimental infection

From the result of a previous LD_{50} experiment (Mostafa, 2007), one dose higher than the LD_{50} dose (6.9×10^7 CFU/fish), around 10^7 CFU/fish was planned as an estimated dose of the pathogen for the present experimental histopathology. Accordingly, for intramuscular (IM) injection, a total of 15 fish were injected intramuscularly with 0.2 ml of a suspension containing 6.9×10^7 CFU/fish of *A. hydrophila* just below the anterior part of the dorsal fin. For intraperitoneal (IP) injection, same numbers of fish were injected intraperitoneally with the same amount of the same suspension just near the pectoral fin carefully. All the injected fish were released into an aquarium and observed for 15 days.

Sampling and follow-up of experimental infection

Water recirculation and aeration were continuously maintained and no feed was given to the fish during the experimental periods. The fish were observed every day. Infection was recorded by observation of lesion, clinical appearance and mortality. Moribund fish were attended, waited for their death and immediately after death transferred to the laboratory for histopathological study of liver, intestine and kidney.

Histological procedure

Collected samples of liver, intestine and kidney were preserved and fixed in 10% neutral buffered formalin. After at least 8 hours of fixation, the samples were trimmed in order to obtain a size of 1 cm^3. The samples were then processed in an automatic tissue processor

(SHANDON, CITADEL 1000) for dehydration, clearing and infiltration. They were then embedded in paraffin wax and sectioned at 5 µm by a microtome (Leica JUNG RM 2035). The ribbon with the sections was placed on a water bath (Electro thermal, MOUNTING BATH) at a temperature of 40°C, which were finally picked up over glass slides. The sections were then stained with haematoxyline and eosin stains and were mounted with Canada balsam. The stained slides were then examined under a compound microscope (Olympus). Photomicrographs of the stained sections were done by using a photomicroscope (OLYMPUS, Model CHS, Japan).

RESULTS

Gross pathological changes

After the challenge test by *A. hydrophila,* the experimental shing fish were observed to loss their normal appearances. Skin colours were also lost and fin erosions were observed in the body of shing. Large external ulcerative lesions were also developed at the injected area. After 2-5 days of injection, petechiae and reddening of the abdomen were noticed in the fish.

Histopathological changes

Histology of liver

Liver of normal shing fish contained no pathology. In case of the challenged fish, the liver developed internal tissue abscess characterized by focal necrosis and hemorrhage in the center of the morbid process (Fig. 1). The distribution of bacterial cells all over the hepatic tissue caused massive diffused necrosis represented by Vacuolation and atrophy (Fig. 2). Degeneration and atrophy of hepatic sinusoids occurred in the liver that were represented by uncoordination of the sinusoidal epithelial layer (Fig. 3). Hamorrhages were also observed in the degenerated and atrophied tissue (Fig. 4).

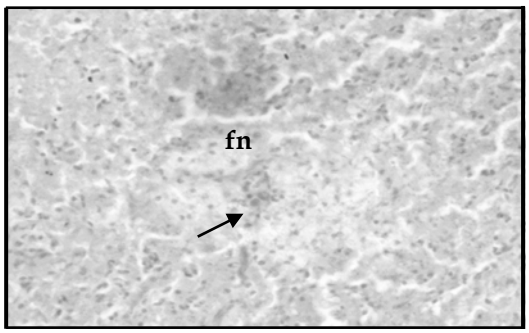

Fig. 1. Hepatic abscess formation characterized by focal necrosis (fn) and hemorrhage in the center of the morbid process (↗). H & E (× 420).

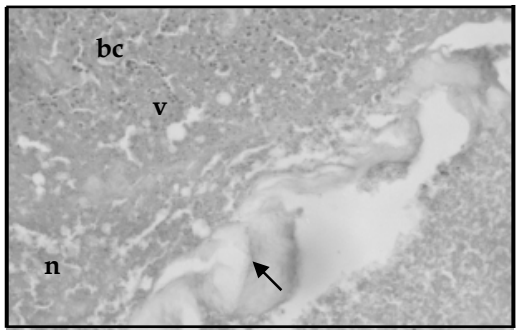

Fig. 2. Dispersion of suspected bacterial colony (bc) in the hepatic tissue together with massive atrophy (↖), necosis (n) and vaculation (v). H & E (× 420).

Histology of kidney

In normal hematopoietic tissue of shing no pathological changes were found but diffuse necrosis occurred in the hemapoietic tissue together with massive atrophy of

hematopoietic sinusoids of the experimental fish. Surrounding the sinusoids there appeared fibrinoid deposition (Fig. 5). Bacterial cells were found to be distributed in the whole hematopoietic tissue including the renal tubules (Fig. 6).

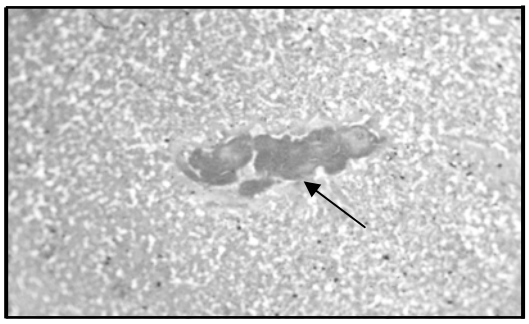

Fig. 3. Degeneration and atrophy of hepatic sinusoids (↖) represented by uncoordination of the sinusoidal epithelial layer. H & E (× 125).

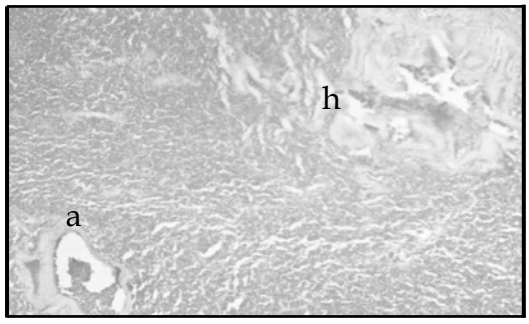

Fig. 4. Hemorrhages (h) in the degenerated and atrophied hepatic tissue (a). H & E (× 125).

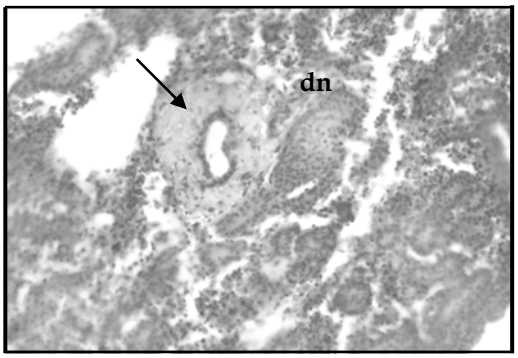

Fig. 5. Massive diffuse necrosis (dn) of the hematopoietic tissue together with massive atrophy of hematopoietic sinusoids and surrounding the sinusoids there appeared fibrinoid deposition (↘). H & E (× 420).

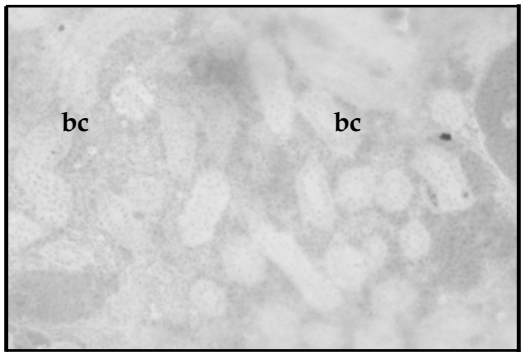

Fig. 6. Suspected bacterial cells (bc) distributed in the whole hematopoietic tissue of infected shing including the renal tubules. H & E (× 420).

Histology of intestine

Normal shing intestine contained no pathology and the epithelium and villi were in normal arrangement. Villi missing and epithelium missing were found in the infected shing (Fig. 7). Hemorrhages and atrophy were found in the epithelial layer of intestine of moribund fish due to the abundance of *A. hydrophila*. Vacuolation and necrosis were found in the epithelial layers that were followed by hemorrhages. Infected fish showed disintegration of *tunica propria* region. In some cases massive atrophy and hemorrhages were also found in *tunica propria* region (Fig. 8).

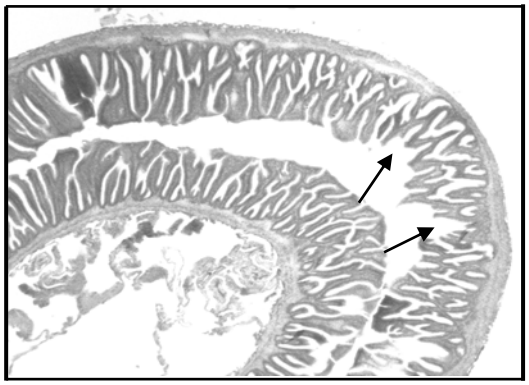

Fig. 7. Villi missing (↘) occurred in the intestine of infected shing. H & E (× 60).

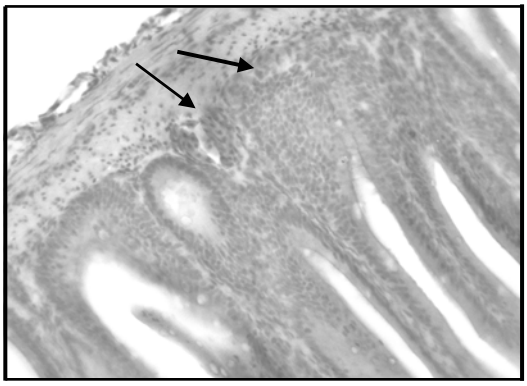

Fig. 8. Hemorrhages (↘) in the *tunica propria* region of the intestinal epithelium. H & E (× 420).

DISCUSSION

Experimental infection by *A. hydrophila* in shing resulted in 85% mortality by a suspension of 6.9×10^7 CFU/fish, with peak mortalities occurring on post infection days 14 and 15 that were similar with the experimental result of Angka (1990) with *A. hydrophila* in *Clarias batrachus* causing 93% mortality by 10^7 CFU/fish. In the present study, large external ulcerative lesions were developed at the injected area represented by reddening and haemorrhages. This type of pathology was also found by Angka (1990) and Panpit-Supap (1988). They found necrotic lesions of the skin and underlying musculature accompanied by inflammation and haemorrhage in walking catfish injected intramuscularly with *A. hydrophila*.

Histologically, Angka (1990) found necrosis and hemorrhage in the kidney, liver, pancreas and intestine that were mostly similar with pathology of liver, kidney and intestine observed in the present study. *A. hydrophila* caused serious pathology in the kidney of the experimental fish. Focal necrosis occurred in haematopoietic tissue together with hemorrhage and atrophy. Afifi (1996) found marked pathological changes in the haematopoietic tissue and vacuolation of the tubular epithelium which contained yellow pigment in mud catfish (*Clarias mossambicus*) experimentally infected with *Trypanosoma markewitschi*. Ahmed and Hoque (1999) reported that histopathologically the internal organs like kidney and liver were more affected by EUS occurred during the months of December and January. Ahmed *et al.* (2004) found EUS related pathology together with Monogenea and Protozoa more in *Channa punctatus* than *Nandus nandus*. Ahmed *et al.* (2006) found that skin, muscle, gill, liver and kidney of *Barbodes gonionotus* showed more pathology in November and December than July to October. Ahmed *et al.* (2007) also found characteristic pathology of EUS in *Anabas testudineus* together with protozoan parasites in December and January. Day *et al.* (1988) observed the presence of *Myxobolus* cysts in the kidney of *Catla* that resulted in degeneration and necrosis of renal tubules and

glomeruli. Presence of mycotic granuloma was also reported in organs like musculature, kidney, spinal cord and other organs of dwarf gourami due to *Aphanomyces* infection by Hatai *et al.* (1994). Mamnur Rashid *et al.* (1997) injected *Edwardsiella tarda* artificially in Japanese flounder and found that bacteria were scattered throughout the kidney tissue except the renal tubules. The authors recommended that this might be due to the septicemic nature of *E. tarda* bacteria. But in this experiment the bacteria *A. hydrophilla* entered in renal tubules as well. This might be due to the invasiveness of *A. hydrophila* unlike *E. tarda.* In the present study, it was found that kidney tubules were swollen, ruptured and missing in many places. Branson and Nieto (1991) reported that kidneys and livers were swollen and grey in colour in farmed coho salmon as also found by Ahmed *et al.* (2004; 2006 and 2007).

Major pathological changes in the present study included massive hepatic atrophy, haemorrhages, necrotic hepatocytes, focal necrosis and atrophy of hepatic sinusoids. *A. hydrophila* bacteria were distributed all over the hepatic tissue. Kumar *et al.* (1991) found highly significant pathological changes in the skin, muscle, kidney, liver and heart of EUS affected *Puntius, Mastacembelus* and *Channa* in India. Hepatocytes were severely necrotic and many empty spaces were created as a result of cell missing. Taveekijakaran *et al.* (1996) observed focal atrophied hepatocytes with dilated sinusoids, multifocal necrosis, perivasculitis and pericholangitis in Amago salmon *Oncorhynchus rhodurus*. Sedano *et al.* (1996) found hypertrophy of intestinal epithelium of infected sea bream larvae which were similar with the pathology of intestine observed in the present study.

It was evident from the experiment that *A. hydrophila* caused gradual pathological symptoms which became severe and massive at the moribund stage of the experimental fish. It proved that *H. fossilis* might be a susceptible fish towards the suspective attack of the ubiquitous bacteria *A. hydrophila* at least in their intensive culture condition. It was pointed out earlier that suspective EUS like ulcerative syndrome were encountered in the Agro-3 farm of Mymensingh area (Hasan, 2007), from where *Aeromonas* bacteria could be isolated. So, it may be concluded that shing fish culturists should have been taken sufficient preventive measure so that their culture environment may be excluded with at least such an ubiquitous opportunistic pathogen like *A. Hydrophila.*

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